2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of hexachlorocyclohexane (HCH). It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure—inhalation, oral, and dermal; and then by health effect—death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods—acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in

determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of hexachlorocyclohexane are indicated in Tables 2-1 and 2-2 and Figures 2-1 and 2-2.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for hexachlorocyclohexane. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

HCH exists as several isomers. The four major isomers discussed in this profile are alpha-HCH (α-HCH), beta-HCH (β-HCH), gamma-HCH (γ-HCH), and delta-HCH (δ-HCH). γ-HCH is also commonly known as lindane. Technical-grade HCH consists of at least 5 isomers (approximately 60–70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH). The toxicity of the isomers varies. With respect to acute exposure, γ-HCH is the most toxic, followed by α-, δ-, and β-HCH. With chronic exposure, however, β-HCH is the most toxic followed by α-, γ-, and δ-HCH. With chronic exposures, the increased toxicity of β-HCH is probably due to its longer biological half-life in the body and its accumulation in the body over time.

2.2.1 Inhalation Exposure

Studies examining the inhalation toxicity of HCH in humans are limited. Most of the available information is from case reports of acute poisoning in the home following the use of γ-HCH vaporizers, whereby γ-HCH pellets are vaporized by electrical warming of a ceramic jacket, and from studies of workers engaged in the manufacture and formulation of pesticides and fertilizers. Limitations inherent in these reports or studies include unquantified exposure concentrations and concomitant exposure to HCH mixtures, pyrolysis products from vaporizers, and other pesticides and chemicals. Studies that provide levels of significant exposure for inhalation exposure to γ-HCH are shown in Table 2-1 and Figure 2-1.

2.2.1.1 Death

γ-HCH was once used in vaporizers, resulting in human exposure to unspecified levels via inhalation and dermal routes. Occasional deaths associated with the use of this product for several months or years have been
reported, but in no case is it clear that γ-HCH was responsible for the deaths (Loge 1965). No human deaths from inhalation exposure to other isomers have been reported.

An acute study with rats exposed to nose-only inhalation of lindane aerosol for 4 hours, followed by a 22-day observation period, estimated the acute LC_{50} to be 1,560 mg/m^3 (Ullmann 1986b). Rats inhaling up to 603 mg/m^3 lindane aerosol for 4 hours in whole-body exposure chambers exhibited no mortality throughout the 14-day observation period (Oldiges et al. 1980). However, the particle sizes produced in aerosol studies are variable, and there is a potential for dermal and oral exposures since the animals could lick their fur.

TABLE 2-1. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation

Key to figure[a]	Species (strain)	Exposure duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (Wistar)	4 hr				1560 (LC_{50})	Ullmann 1986b
2	Mouse (CD-1)	1 wk 5 d/wk 6 hr/d				10 (16% mortality)	Klonne and Kintigh 1988
Systemic							
3	Rat (Wistar)	4 hr	Resp	603			Oldiges et al. 1980
			Hepatic	603			
			Renal	603			
Neurological							
4	Rat (Wistar)	4 hr			101 (sedation)	642 (restlessness, excitation, ataxia)	Ullmann 1986b
INTERMEDIATE EXPOSURE							
Death							
5	Mouse (CD-1)	14 wk 5 d/wk 6 hr/d				1.0 (2% mortality)	Klonne and Kintigh 1988

Figure 2-1. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation (cont.)

Intermediate (15-364 days)

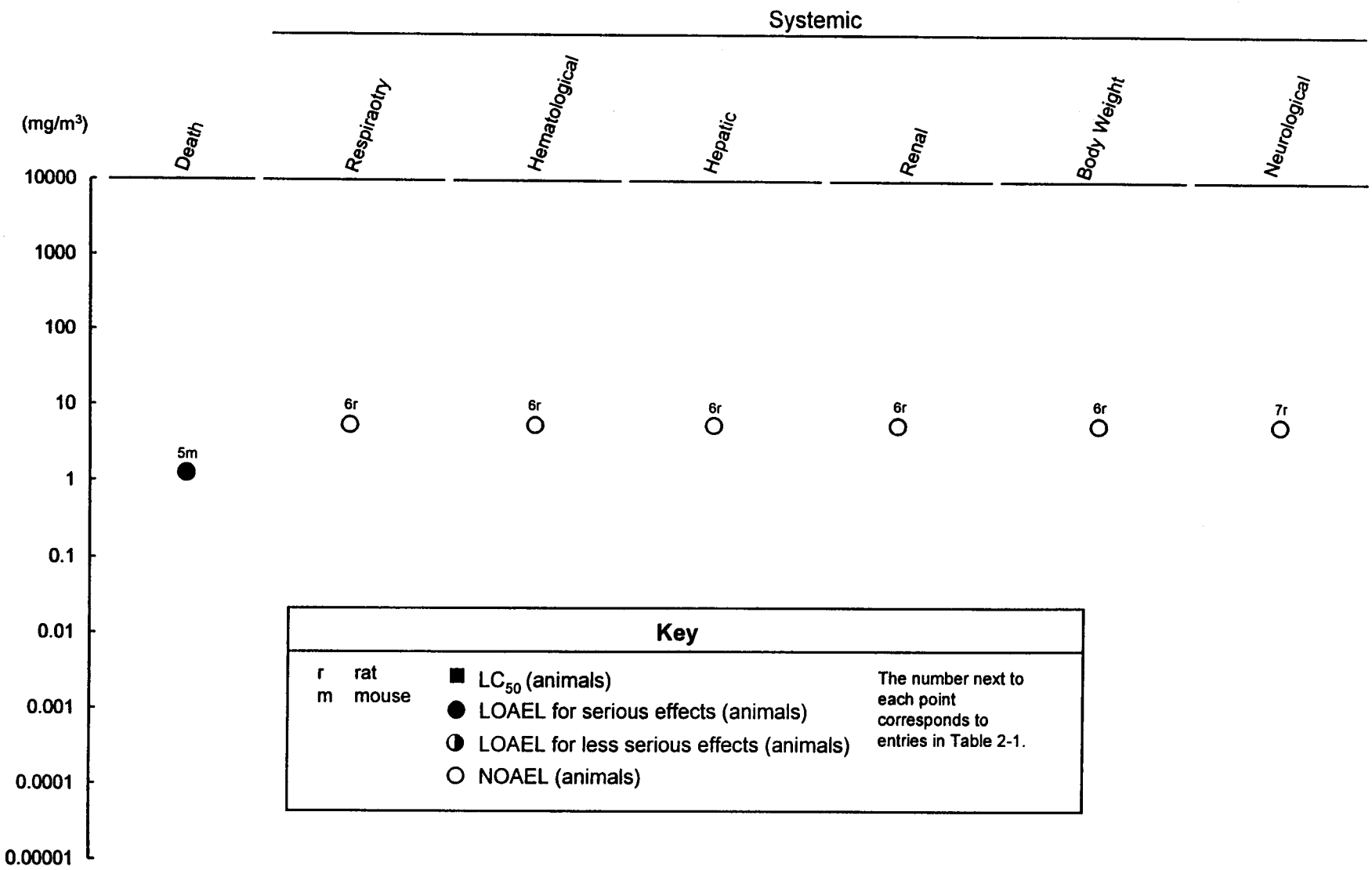


TABLE 2-1. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference
					Less serious (mg/m3)	Serious (mg/m3)	
Systemic							
6	Rat (Wistar)	90 d 6 hr/d	Resp	5			Oldiges et al. 1983
			Hemato	5			
			Hepatic	5			
			Renal	5			
			Bd Wt	5			
Neurological							
7	Mouse (CD-1)	14 wk 5 d/wk 6 hr/d		5			Klonne and Kintigh 1988

[a]The number corresponds to entries in Figure 2-1.

Bd Wt = body weight; d = day(s); Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s).

Figure 2-1. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation

Acute (≤14 days)

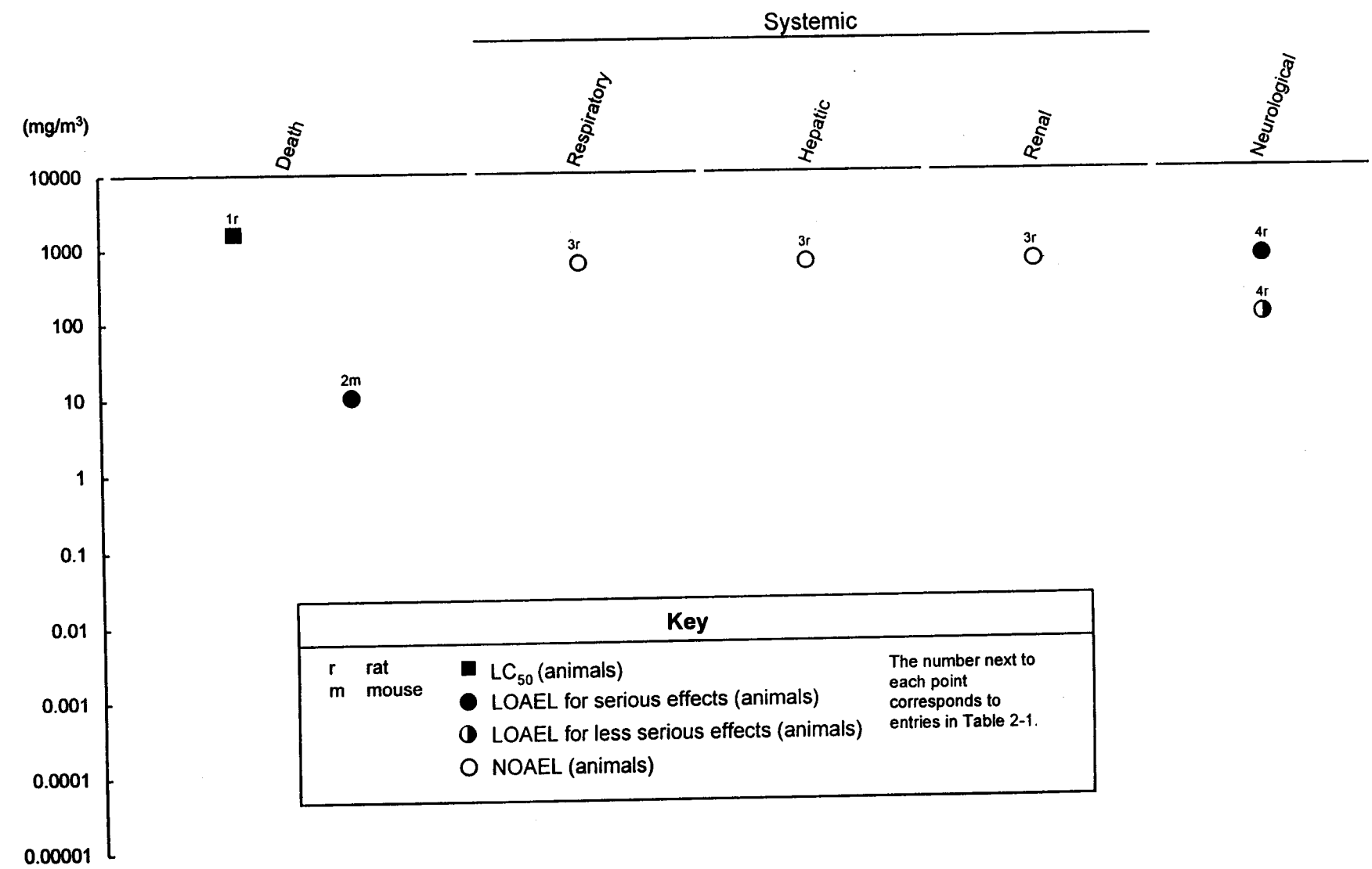
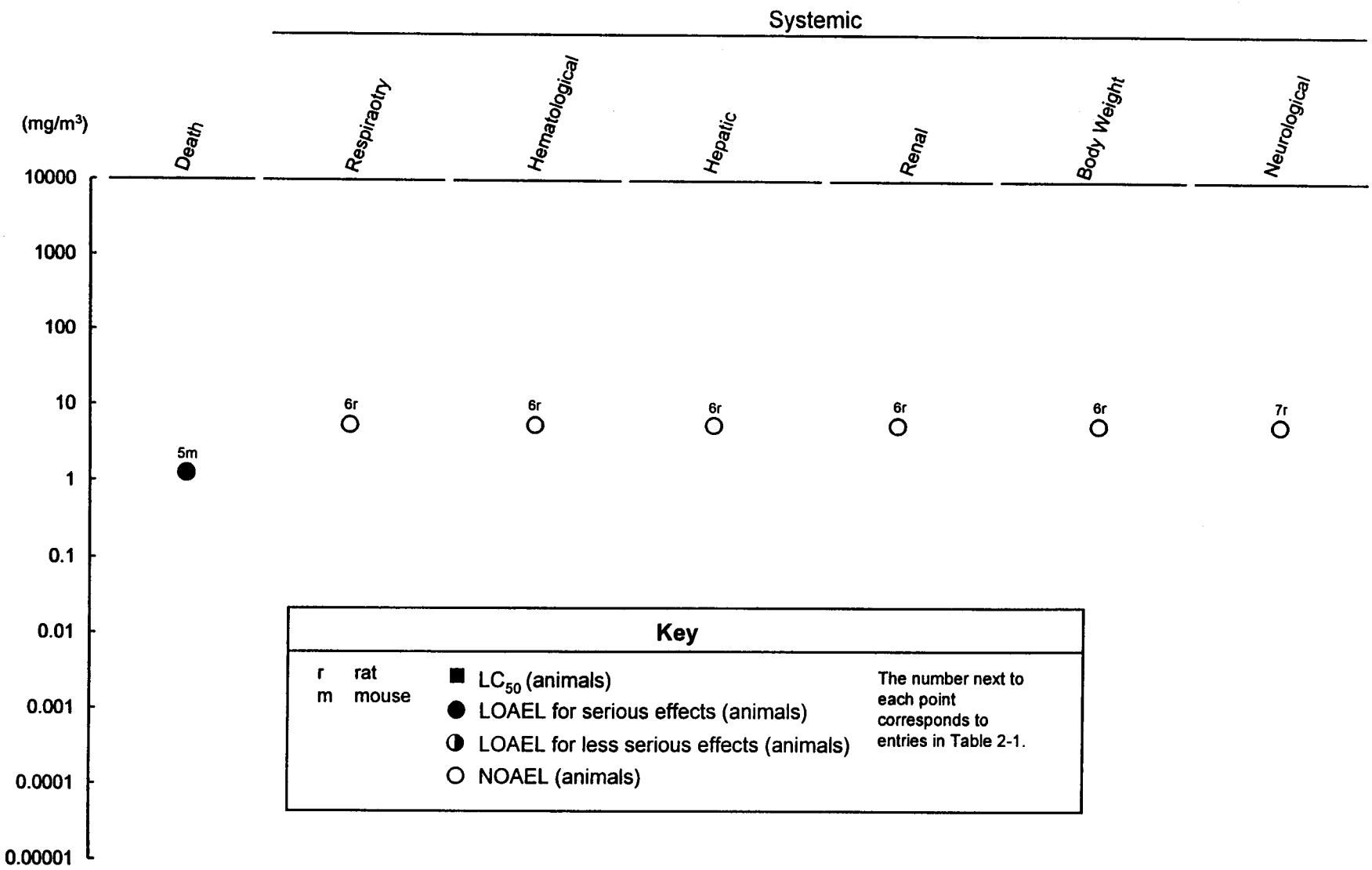


Figure 2-1. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation (cont.)

Intermediate (15-364 days)



Therefore, the estimated doses delivered to the animals cannot be precisely determined, and thus, the toxicity levels cited may be of questionable validity. In an intermediate-duration study with mice inhaling lindane dust aerosol in whole-body exposure chambers, 16% mortality was observed after 1 week of exposure to 10 mg/m^3, while exposures of up to 14 weeks resulted in 22% mortality at 5 mg/m^3, 2% mortality at 1 mg/m^3, and no mortality at 0.3 mg/m^3 (Klonne and Kintigh 1988).

2.2.1.2 Systemic Effects

Respiratory Effects. In humans, mucous membrane irritation of the nose and throat was observed after acute exposure to the HCH products dispensed by an overheated γ-HCH vaporizer (Conley 1952). Exposure levels were not reported and dermal exposure may also have occurred, although the observed irritation was probably due to direct action upon the mucous membranes.

No respiratory effects were observed in rats exposed to up to 603 mg/m^3 lindane aerosol for 4 hours (Oldiges et al. 1980). No respiratory effects were observed in rats exposed to lindane aerosol (up to 5 mg/m^3) for 90 days (Oldiges et al. 1983) or in mice similarly exposed for 14 weeks (Klonne and Kintigh 1988).

Cardiovascular Effects. Cardiovascular effects of HCH have been reported in humans exposed to HCH. Kashyap (1986) reported electrocardiogram (ECG) abnormalities in 15% of 45 factory workers involved in the production of technical-grade HCH; exposure concentrations were not reported and dermal exposure may have occurred.

No studies were located regarding cardiovascular effects in animals following inhalation exposure to HCH.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans or animals following inhalation exposure to HCH.

Hematological Effects. Hematological effects have been reported in humans following acute or chronic inhalation exposure to γ-HCH; however, a causal relationship between exposure to γ-HCH and hematological effects in humans has not been established. Hypochromic anemia was reported in a 2.5-year-old boy who was exposed to γ-HCH in a home in which a pesticide vaporizer was operated. Air γ-HCH concentrations measured in the basement and living room of the house were 2.4–5.5 $\mu g/m^3$; however, the actual concentration the child was exposed to and the duration of exposure were not determined (Morgan

et al. 1980). Aplastic anemia was reported in a boy exposed to γ-HCH used as an insecticide in his home and in a man exposed at work (Rugman and Cosstick 1990). The anemia was reversible and was not present in other family members. The levels and routes of exposure are not known, although they are presumed to be inhalation and dermal. Other hematological abnormalities, including isolated instances of leukopenia, leukocytosis, granulocytopenia, granulocytosis, eosinophilia, monocytosis, and thrombocytopenia, have been reported following chronic human occupational exposure to γ-HCH (Brassow et al. 1981; Jedlicka et al. 1958). Exposure concentrations were not specified in these studies and concomitant dermal exposure probably occurred. Although Brassow et al. (1981) reported slight changes in clinical chemistry tests in 60 human workers, there were no cases of severe impairment of health. Granulocytopenia, aplastic anemia, paramyeloblastic leukemia, and pancytopenia have been reported in a number of case reports of individuals following exposure to γ-HCH and other pesticides such as DDT in the home, during the handling of the pesticide, or from a nearby formulating plant (Danopoulos et al. 1953; Friberg and Martensson 1953; Gewin 1939; Loge 1965; Mendeloff and Smith 1955). Exposure concentrations were not reported, dermal exposure was likely, and in many cases there was concomitant exposure to other pesticides; therefore, determination of a causal relationship between exposure and hematological effects cannot be made.

No hematological effects were seen in rats exposed to lindane aerosol (up to 5 mg/m^3) for 90 days (Oldiges et al. 1983).

Hepatic Effects. In humans, statistically significant increases in the blood levels of the enzymes lactate dehydrogenase (33%), leucine aminopeptidase (45%), and γ-glutamyl transpeptidase (174%) were reported in 19 individuals occupationally exposed to technical-grade HCH for over 10 years in an HCH-formulating plant (Kashyap 1986); the HCH isomer concentrations showed a 1-fold increase compared to the control group of workers. Both inhalation and dermal exposure probably occurred. The large standard deviation (SD) from the mean reported for γ-glutamyl transpeptidase in exposed workers (mean±SD = 22.2±40.31 25 μ/mL) suggests the increased level of this enzyme may not be related to HCH exposure or that individual responses may vary.

No hepatic effects were observed in rats after acute exposure to 603 mg/m^3 γ-HCH (Oldiges et al. 1980). Rats exposed to lindane aerosol (5 mg/m^3) exhibited increased hepatic cytochrome P-450 concentration after 90 days, but this level returned to control values after a 4-week recovery period (Oldiges et al. 1983).

Renal Effects. No studies were located regarding renal effects in humans following inhalation exposure to HCH.

No renal effects were seen in rats exposed to up to 603 mg/m^3 lindane aerosol for 4 hours (Oldiges et al. 1980) or up to 5 mg/m^3 lindane aerosol for 90 days (Oldiges et al. 1983).

Endocrine Effects. Serum luteinizing hormone levels which were reported to be statistically significant, increased in 54 men occupationally exposed to γ-HCH for approximately 8 years in a γ-HCH producing factory (Tomczak et al. 1981). The mean serum concentration of follicle stimulating hormone was increased and testosterone was decreased; but these differences were not statistically significant (Tomczak et al. 1981).

No studies were located regarding endocrine effects in animals following inhalation exposure to HCH.

Dermal Effects. No studies were located regarding dermal effects in humans or animals following inhalation exposure to HCH.

Ocular Effects. No studies were located regarding ocular effects in humans following inhalation exposure to HCH.

Mice exposed to lindane aerosol (up to 5 mg/m^3) for 14 weeks exhibited no ophthalmic effects (Klonne and Kintigh 1988).

Body Weight Effects. No studies were located regarding body weight effects in humans following inhalation exposure to HCH.

No body weight effects were seen in rats exposed to up to 5 mg/m^3 lindane aerosol for 90 days (Oldiges et al. 1983).

2.2.1.3 Immunological and Lymphoreticular Effects

A statistically significant increase (approximately 18%) in the level of immunoglobulin M (IgM) was noted in 19 workers occupationally exposed to technical-grade HCH during pesticide formulation as compared to 14 nonexposed workers (Kashyap 1986). The HCH isomer concentrations in serum showed a 10-fold

increase when compared to the control group. Both inhalation and dermal exposure probably occurred, and the measurement of IgM alone is not a reliable measure of immune function in adults.

No studies were located regarding immunological or lymphoreticular effects in animals following inhalation exposure to HCH.

2.2.1.4 Neurological Effects

Paresthesia of the face and extremities, headache, and vertigo have been reported in a group of 45 workers occupationally exposed during manufacture and formulation of technical-grade HCH for several years (Kashyap 1986); exposure concentrations were not reported. Both inhalation and dermal exposure probably occurred. Abnormal electroencephalographic (EEG) patterns (increased variation in the frequency and amplitude of wave pattern or more serious changes without specific EEG signs) have been reported in 16 of 37 workers following exposure to γ-HCH for 0.5–2 years in a fertilizer plant (Czegledi-Janko and Avar 1970). Exposure concentrations were not reported; however, these EEG changes were found to correlate with blood levels of γ-HCH. Weakness of the left and right limbs, dysarthria, and dysphagia were seen in an agricultural worker exposed by inhalation and dermal contact to unspecified levels of several organochlorine pesticides, including lindane (Fonseca et al. 1993).

Rats exposed to various concentrations of 99.6% lindane aerosol via nose-only inhalation for 4 hours exhibited dose-related neurological effects when observed for up to 22 days after exposure (Ullmann 1986b). Slight-to-moderate sedation was observed after exposure to 101 mg/m^3; slight-to-severe sedation was noted after exposure to 378 mg/m^3; restlessness, excitation, and ataxia were seen after exposure to 642 and 2,104 mg/m^3; and spasms were also noted at the highest concentration (2,104 mg/m^3). Rats exposed to 0.02–5 mg/m^3 lindane aerosol for 90 days exhibited a "slightly disturbed general condition" beginning at day 15 (Oldiges et al. 1983). Mice were similarly exposed for 14 weeks and exhibited no clinical signs of neurotoxicity (Klonne and Kintigh 1988).

2.2.1.5 Reproductive Effects

Statistically significant increases in the levels of serum luteinizing hormone were reported in a group of 54 men occupationally exposed to γ-HCH for approximately 8 years in a γ-HCH-producing factory (Tomczak et al. 1981). Although the mean serum concentration of follicle stimulating hormone was

increased and testosterone was decreased, these differences were not statistically significant. No causal relationship could be established because exposure levels were not reported. These hormonal changes may have resulted in diminished reproductive capability.

No studies were located regarding reproductive effects in animals following inhalation exposure to HCH.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals following inhalation exposure to HCH.

2.2.1.7 Genotoxic Effects

No increase in the frequency of chromosome aberrations was observed in humans exposed primarily to γ-HCH by inhalation in a pesticide production factory (Kiraly et al. 1979). These individuals had been exposed for 8 hours/day for at least 6 months. Other studies are available regarding genotoxic effects in humans exposed to a wide variety of pesticides, including lindane, when they were used on farms (Rupa et al. 1988, 1989a, 1989b, 1989c). The specific effects of HCH, apart from the effects due to the other exposures, are not known.

No studies were located regarding genotoxic effects in animals following inhalation exposure to HCH.

Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

Use of γ-HCH pesticides by farmers in 4 western or midwestern states was associated with a 50% increased risk of having non-Hodgkin's lymphoma (Blair et al. 1998). There was some evidence of a nonstatistically significant dose-response relationship because odds ratios (OR) were greater in farmers who used γ-HCH pesticides $20 compared with <20 years prior to diagnosis (OR 1.7 compared with 1.3) and $5 compared with <5 times per year (OR 2.0 versus 1.6). However, use of certain insecticides such as 2,4-D and diazinon reduced odds ratios from 1.5 to 1.2 and 1.3, respectively. The authors concluded that γ-HCH is not a major factor in the development of non-Hodgkin's lymphoma but may play some role.

No studies were located regarding carcinogenic effects in animals following inhalation exposure to HCH.

2.2.2 Oral Exposure

There are two tables and two figures for the Levels of Significant Exposure for oral exposure to the HCH isomers. Table 2-2 and Figure 2-2 are for γ-HCH. Table 2-3 and Figure 2-3 are for α-, β-, and δ-HCH, and technical-grade HCH.

2.2.2.1 Death

Occasional deaths of humans (usually children) have been reported following ingestion of γ-HCH, often from the tablets intended for γ-HCH vaporizers (Storen 1955). γ-HCH has also been used for suicide (Sunder Ram Rao et al. 1988). The levels associated with death are not known.

γ-HCH has been shown to be lethal to animals following single gavage administration (Gaines 1960; Liu and Morgan 1986; Tusell et al. 1987). The LD_{50} value for female rats is 91 mg/kg, and the LD_{50} value for male rats is 88 mg/kg (Gaines 1960). One of 7 male Wistar rats died following a single oral administration of 60 mg/kg γ-HCH (Martinez et al. 1991). DBA/2 strain mice, recognized as being "unresponsive" to microsomal enzyme induction, are more sensitive to the acute lethal effects of γ-HCH than C57BL/6 strain mice when exposed to 20 mg/kg/day for 10 days (Liu and Morgan 1986). In a 15-week study, 2 of 12 F-344 rats treated with 20 mg/kg/day died (Chadwick et al. 1988). A 2-year study in rats fed lindane in their diets (32 mg/kg/day) also found a significantly increased mortality rate compared with controls (Amyes 1990). The oral LD_{50} for technical-grade HCH in CFT-Wistar rats treated once by gavage was 2,428 mg/kg (Joseph et al. 1992a). Exposure to 5 mg/kg/day of technical-grade HCH for 90 days resulted in the deaths of 6/12 male rats and 4/12 female rats (Dikshith et al. 1991b). Exposure to low levels (0.4 mg/kg/day) of technical-grade HCH in the diet for 360 days resulted in deaths of 4/20 rats (Dikshith et al. 1991a). However, the deaths occurred late in the study and were accompanied by other changes indicating that they were due to pathogenic infection rather than HCH exposure.

The LD_{50} for rats and the LOAEL values from the intermediate-duration studies are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-2 and 2-3.

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (Sherman)	once (GO)				88 M (LD_{50}) 91 F (LD_{50})	Gaines 1960
2	Rat (Wistar)	once (GO)				60 M (1/7 deaths)	Martinez et al. 1991
Systemic							
3	Rat	2wks (F)	Hepatic		72 Altered activities of serum aminotransferases, alkaline phosphatase, decreased soluble enzymes and altered carbohydrate metabolism.		Srinivasan and Radhakrishnamurty 1988
4	Rat (Wistar)	14 d ad libitum (F)	Renal			72 M (10% increase in kidney weight, altered excretion patterns, distention of glomeruli, swelling of tubular epithelia)	Srinivasan et al. 1984

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
5	Mouse (B6C3F1)	3 d 1x/d (GO)	Resp	40 M			Hong and Boorman 1993
			Cardio	40 M			
			Gastro	40 M			
			Hemato		20M (Transient reduction in marrow progenitor cell number)		
			Hepatic	40 M			
			Renal	40 M			
			Endocr	40 M			
			Bd Wt	40 M			
6	Mouse (B6C3F1)	10 d 1 x/d (GO)	Resp	20 M			Hong and Boorman 1993
			Cardio	20 M			
			Gastro	20 M			
			Hemato		10M (Transient decrease in marrow progenitor cell numbers)		
			Hepatic	20 M			
			Renal	20 M			
			Bd Wt	20 M			
Immunological/Lymphoreticular							
7	Mouse (B6C3F1)				10M (Dose-related decrease in thymus and spleen weights)		Hong and Boorman 1993
8	Mouse (B6C3F1)	3 d 1x/d (GO)		10 M	20M Decreased thymus transient weight	40 M Atrophy of thymus cortex	Hong and Boorman 1993

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
Neurological							
9	Rat (Sprague-Dawley)	6 d 1x/d (GO)			3M (increased pineal *N*-acetyltransferase, decreased serotonin levels)		Attia et al. 1991
10	Rat (Wistar)	once (GO)				30 M (convulsions, decreased calmodulin mRNA expression in the brain)	Barron et al. 1995a
11	Rat (Long- Evans)	once (GO)			5M (myoclonic jerks and single clonic seizure in kindled animals)	10 M (myoclonic jerks and single clonic seizures in naive animals)	Gilbert and Mack 1995
12	Rat (Sprague-Dawley)	4 d 1x/d (GO)		1[b] M	3M (increased kindling acquisition)	10 M (seizures)	Joy et al. 1982
13	Rat (Wistar)	once (GO)				60 (convulsions)	Martinez and Martinez-Conde 1995
14	Rat (Wistar)	once (GO)				60 M (tonic-clonic seizures)	Martinez et al. 1991
15	Rat (Wistar)	3 d 1x/d (GO)			5 (decreased myelin and 2',3'-cyclic nucleotide 3'-phosphodiesterase activity in brains)		Serrano et al. 1990a

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
16	Rat (Wistar)	once (GO)		15 M		20 M (convulsions)	Vendrell et al. 1992a
17	Rat (Sprague-Dawley)	once (GO)				30 M (seizures)	Wooley and Griffith 1989
	Reproductive						
18	Rat	6 days, day 9-14 of lactation			1M (reduced testosterone level at puberty, relative testes weight)		Dalsenter et al. 1997a
19	Rat	once day 9 or 14 of lactation (GO)			6M Reduced relative testical and epidymis weight (~10%), spermatid and sperm counts (~8-10%), testosterone levels (~30-50%), Leydig cell numbers and spermatogenisis.		Dalsenter et al. 1997a
20	Rat (Long- Evans)	7 d 1x/d (GO)		40 F			Laws et al. 1994
21	Rat (CDF-F344)	once			25 (increased length of estrous cycle)		Uphouse and Williams 1989

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL	LOAEL Less serious	LOAEL Serious	Reference
Developmental							
22	Rat (Wistar)	single dose day 15 of gestation (GO)			30 (reduction of serum testosterone concentration in adult offspring)		Dalsenter et al. 1997b
23	Rat (Wistar)	Gd 6-15 1x/d (GO)		25 F			Khera et al. 1979
24	Rat (CFY)	Gd 6-15 1x/d (G)		20 F			Palmer et al. 1978a
25	Rat (Wistar)	once (GO)			20 (regional changes in brain noradrenaline and serotonin levels in suckling rats)		Rivera et al. 1991
26	Mouse DBA/2J	Single oral dose on day 12 of gestation GI			45 (decrease in fetal and placental weight)		Hassoun and Stohs, 1996a
27	Mouse C57BL/6N	Single oral dose on day 12 of gestation GI			30 (decrease in fetal weight, fetal thymus weight)		Hassoun and Stohs, 1996a

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
28	Rabbit (New Zealand)	Gd 6-18 1x/d (G)		20 F			Palmer et al. 1978a
INTERMEDIATE EXPOSURE							
Death							
29	Rat (Fischer-344)	15 wk 1x/d (GO)				20 F (2/12 deaths)	Chadwick et al. 1988
Systemic							
30	Rat (Wistar)	15 d ad libitum (F)	Hepatic		1.8 M Increases in lipid peroxidation, level of cytochrome P-450, and activities of superoxide dismutase.		Barros et al. 1991
31	Rat (Wistar)	30 d ad libitum (F)	Hepatic		1.8 M Increases in lipid peroxidation, level of cytochrome P-450, and activities of superoxide dismutase.		Barros et al. 1991
32	Rat (Wistar)	40d (F)	Hepatic Renal	50	 5		Desi 1974

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL	LOAEL Less serious	LOAEL Serious	Reference
33	Rat (Wistar)	7 and 15 days gavage (SC)	Gastro		20 Reduction in jejunum maltase activity		Moreno et al. 1996
34	Rat (Wistar)	12 wk ad libitum (F)	Hepatic	0.4	2 (centrilobular hypertrophy)		Suter 1983
			Renal	0.4	2 (ddtubular distension, basophilic tubules)		
			Hemato	10			
35	Mouse (dd)	24 wk ad libitum (F)	Hepatic		90M (centrilobular hypertrophy)		Ito et al. 1973
Immunological/Lymphoreticular							
36	Mouse (Swiss albino)	24 wk ad libitum (F)			0.012[c] F (biphasic changes in cell- and humoral-mediated immune system)	1.2 F (necrosis of thymus)	Meera et al. 1992
Neurological							
37	Rat (Wistar)	90 d ad libitum (F)				90 M (tonic convulsions)	Arisi et al. 1994
38	Rat (Long- Evans)	30 d 1x/d (GO)				10 M (myoclonic jerks and clonic seizures)	Gilbert 1995

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
39	Rat (Long- Evans)	10 wk 3 d/wk (GO)				10 M (myoclonic jerks and clonic seizures)	Gilbert 1995
40	Rat (Wistar)	30 d (GO)			2 (decreased dopamine levels)		Martinez and Martinez-Conde 1995
41	Rat (Wistar)	30 d ad libitum (F)		12.3 M	25.4 M (reduced tail nerve conduction velocity)		Muller et al. 1981
	Reproductive						
42	Rat (Fischer-344)	15 wk 1x/d (GO)		5 F	10 F (disrupted ovarian cycling, antiestrogenic effects)		Chadwick et al. 1988
43	Rabbit (hybrid)	12 wk 3 d/wk (GO)			0.8 F (reduced ovulation rate)		Lindenau et al. 1994
44	Rabbit (New Zealand)	12-15 wk 3 d/wk (GO)		0.8 F			Seiler et al. 1994

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
Developmental							
45	Rat (Wistar)	21 day GO 21 GD and 28 LD or 28 LD (F)			25 Increased liver weight and decreased kidney weight in pups exposed during gestatino and lactation		Srinivasan et al. 1991a
46	Rabbit (New Zealand)	12-15 wk 3 d/wk (GO)		0.8 F			Seiler et al. 1994
CHRONIC EXPOSURE							
Death							
47	Rat (Wistar)	up to 52 weeks ad libitum (F)				32 F (increased mortality rate)	Amyes et al. 1990
Systemic							
48	Rat (Wistar)	5 to 52 weeks ad libitum (F)	Hepatic Renal	0.7 M 0.8 F 0.7 M 8 F	7 M (periacinar hepatocytic 8 F hypertrophy) 7 M (male: pale kidneys, 28 F increased kidney weight, urinary volume, and protein, tubular necrosis. female: increase in urine specific gravity, urea, and creatinine and kidney weight)		Amyes et al. 1990

TABLE 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
49	Rat (Wistar)	109 weeks (F)	Hepatic	3.5 M 4.0 F		7 M (focal necrosis, fatty 8 F degeneration, 35% increase in liver weight)	Fitzhugh et al. 1950
			Renal	3.5 M 4 F	7 M (focal nephritis) 8 F		
			Bd Wt	56 M	112M (17% decrease in body weight gain)		
				64 F	128F (13 % decrease in body weight gain)		
Cancer							
50	Mouse (B6C3F1)	80 wk ad libitum (F)				13.6 M (CEL: hepatocellular carcinoma)	NCI 1977
51	Mouse (F-1 hybrid)	24 mo ad libitum (F)				27.2 F (CEL: hepatocellular carcinoma, lung tumors)	Wolff et al. 1987

[a]The number corresponds to entries in Figure 2-2.

[b]Used to derive an acute-duration oral Minimal Risk Level (MRL) of 0.01 mg/kg/day for gamma-HCH; 1 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability) = 0.01 mg/kg/day.

[c]Used to derive an intermediate-duration oral Minimal Risk Level (MRL) of 0.00001 mg/kg/day for gamma-HCH; 0.012 mg/kg/day divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, 10 for human variability) = 0.00001 mg/kg/day.

Bd Wt = body weight; CEL = cancer effect level; d = day(s); F = female; (F) = food; (G) = gavage; (GO) = gavage, oil; (GO) = gavage, water; Gd = gestation day(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mRNA = messenger ribonucleic acid; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s); x = time(s); yr = year(s).

Figure 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral

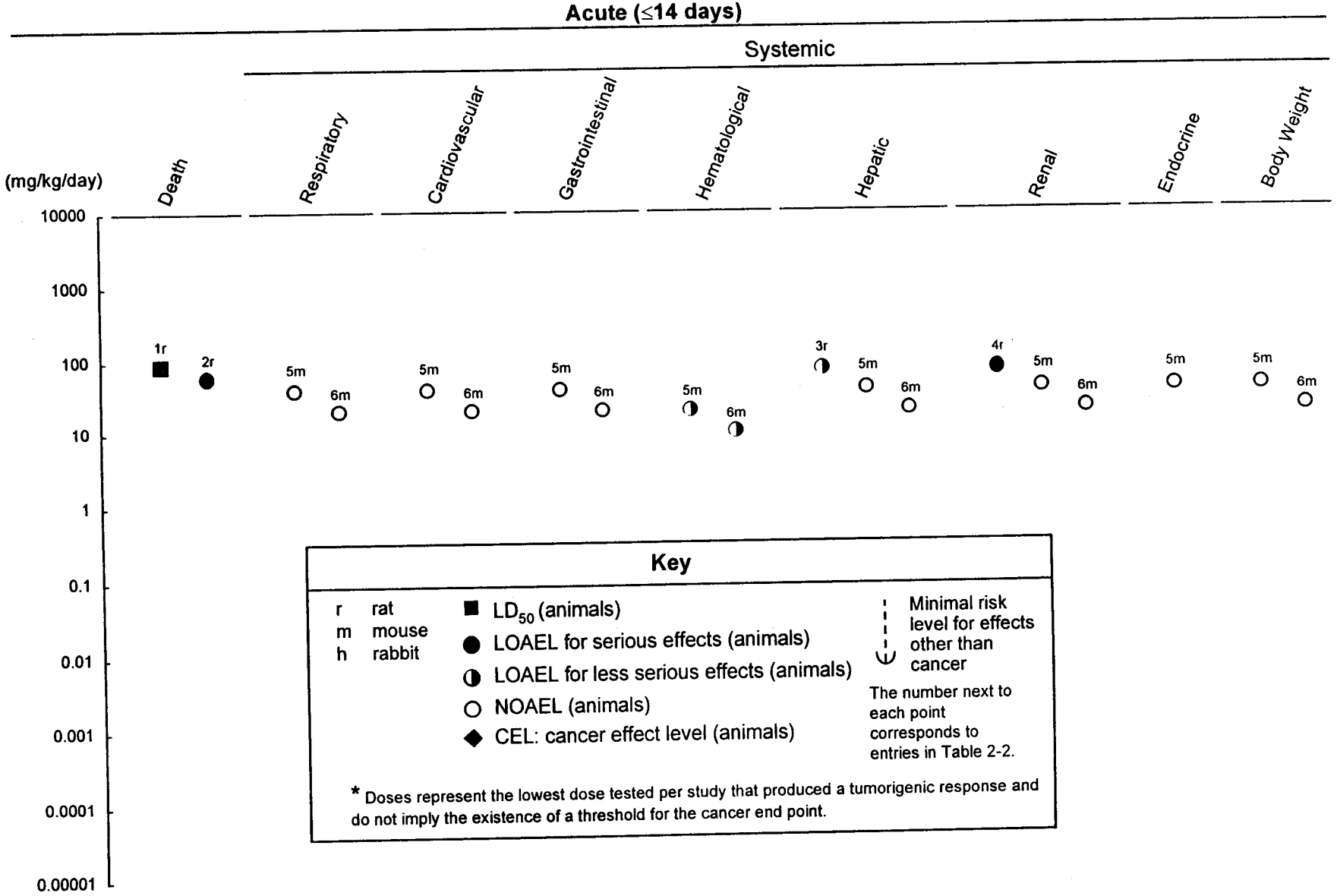


Figure 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (cont.)

Acute (≤14 days)

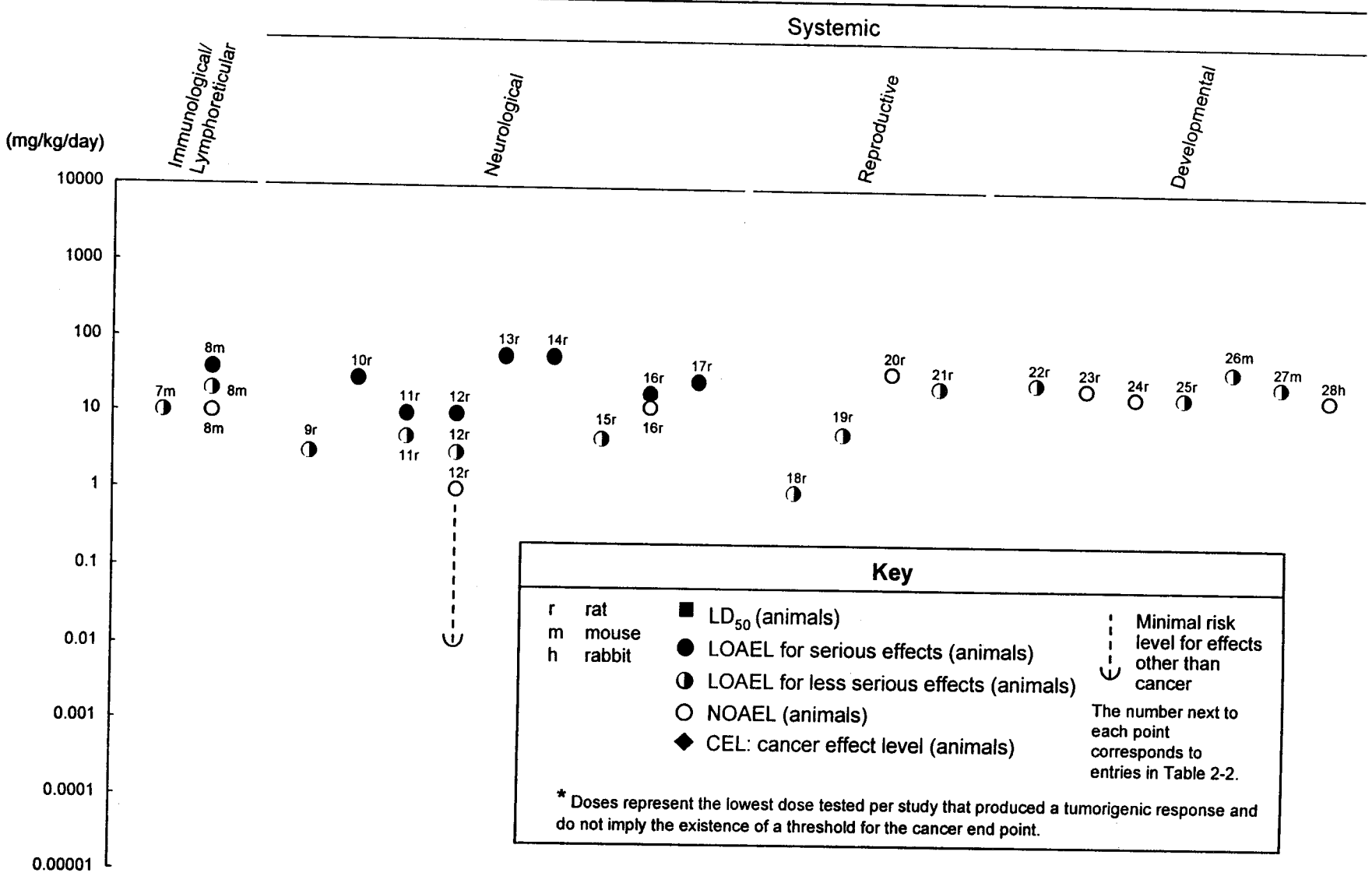


Figure 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (cont.)

Intermediate (15-364 days)

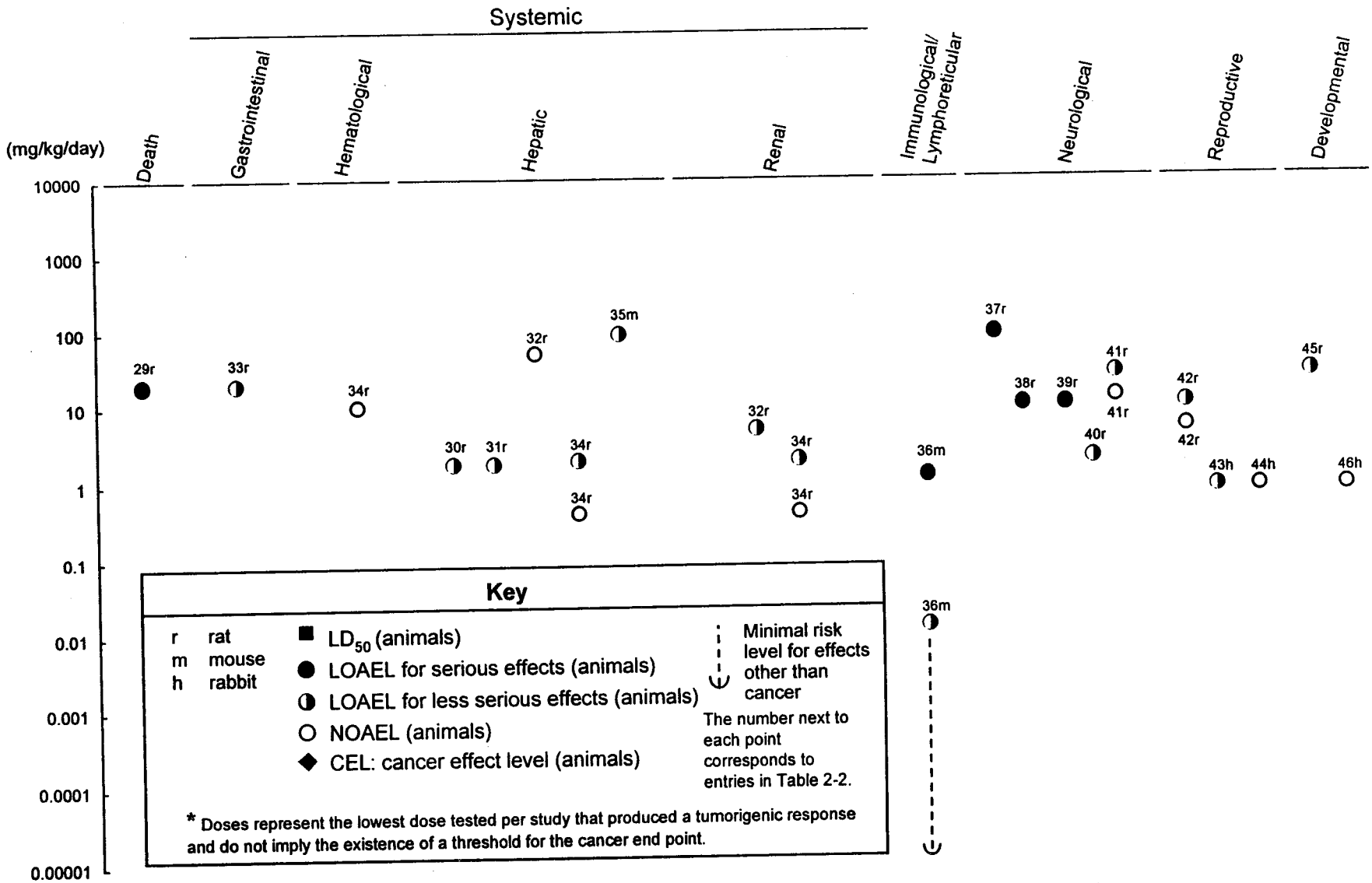


Figure 2-2. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Oral (cont.)

Chronic (≥365 days)

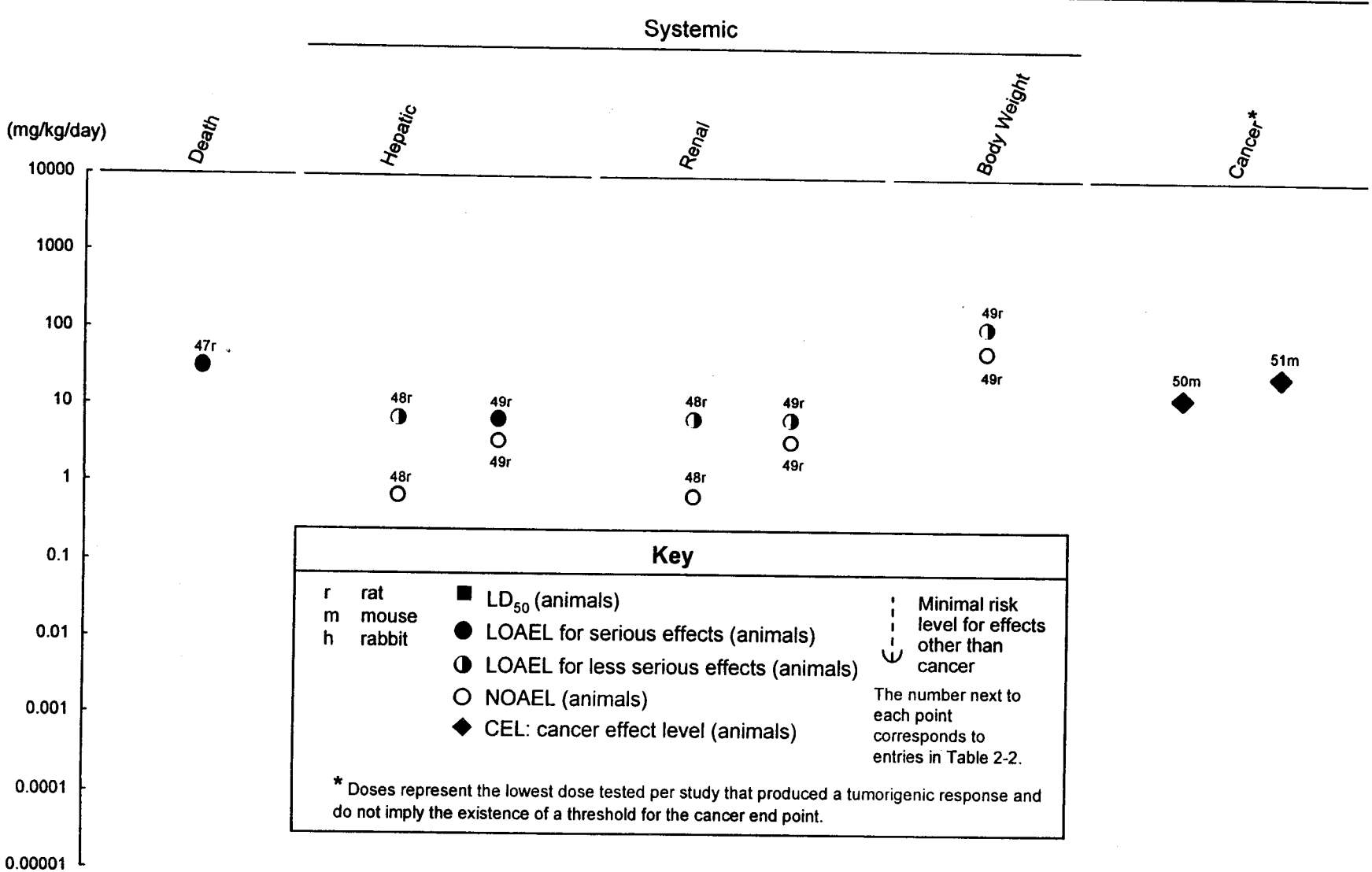


TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference/ Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat (CFT-Wistar)	once (GO)				2428 M (LD_{50})	Joseph et al. 1992a technical
Systemic							
2	Rat (NS)	once (GO)	Metab		100F (increased phosphoinositide turnover in erythrocyte membranes)		Agrawal et al. 1995 technical
3	Rat (Sprague-Dawley)	2 wk ad libitum (F)	Hepatic		90M (increased triglycerides, phospholipids and cholesterol, increased cytochrome C reductase and decreased glutathione peroxidase)		Ikegami et al. 1991a beta
4	Rat (Sprague-Dawley)	2 wk ad libitum (F)	Hepatic		90M (increased relative liver weight and cytochrome P-450 levels and decreased hepatic vitamin A levels)		Ikegami et al. 1991b beta
5	Rat (Wistar)	14 d ad libitum (F)	Renal			72 M (tubular degeneration, distention of glomeruli, swelling of tubular epithelia, 22% increase in kidney weight, altered excretion patterns)	Srinivasan et al. 1984 beta

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
6	Mouse (Swiss albino)	Gd 9 once (GO)	Hepatic		5F (significantly decreased GOT, GPT, and lactate dehydrogenase (LD) activities)		Dikshith et al. 1990 technical
7	Mouse (NS)	1, 5, 15 d 1x/d (GO)	Hepatic			50 (congestion of portal vessels and central vein, fatty changes, granular degeneration)	Philip et al. 1989 technical
			Renal			50 (congestion of portal vessels and glomeruli, fatty changes, interstitial hemorrhaging)	
8	Mouse (Swiss albino)	2 wk ad libitum (F)	Hepatic		72M (226% increase in liver weight, increased serum alanine and aspartate aminotransferases and ALP, increased hepatic phosphatases and acid cathepsin)		Ravinder et al. 1989 technical
9	Mouse (Swiss albino)	2 wk ad libitum (F)	Hepatic		72M (cellular hypertrophy, centrilobular degeneration, focal necrosis)		Ravinder et al. 1990 technical
Neurological							
10	Rat (Wistar)	once (GO)			100M (decreased calmodulin mRNA expression in the brain)		Barron et al. 1995 delta
11	Mouse (B6C3F1)	1 wk ad libitum (F)		19[b] F	57F (ataxia)	190 F (lateral recumbancy)	Cornacoff et al. 1988 beta

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
Reproductive							
12	Mouse (Swiss albino)	Gd 9 once (GO)		5 F		25 F (increased fetal resorptions)	Dikshith et al. 1990 technical
INTERMEDIATE EXPOSURE							
Death							
13	Rat (NS)	360 d ad libitum (F)				0.4 M (4/20 deaths)	Dikshith et al. 1991a technical
14	Rat (NS)	90 d 1x/d (GO)				5 (6/12 M, 4/12 F died)	Dikshith et al. 1991b technical
Systemic							
15	Rat (NS)	3-6 mo 5 d/wk (GO)	Metab		5F (increased phosphoinositide turnover in erythrocyte membranes and cerebrum)		Agrawal et al. 1995 technical
16	Rat (Wistar)	30 d ad libitum (F)	Hepatic		1.8 M (increased cytochrome P-450 level, superoxide dismutase, catalase, NADPH-cytochrome P-450 reductase activities, and lipid peroxidation)		Barros et al. 1991 alpha

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference/ Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
17	Rat (Wistar)	15 d ad libitum (F)	Hepatic		1.8 M (increased cytochrome P-450 level, superoxide dismutase, catalase, and lipid peroxidation activities)		Barros et al. 1991 alpha
18	Rat (NS)	30 d 1x/d (GO)	Hemato	60 M			Dikshith et al. 1989a technical
			Hepatic		60 M (decreased GOT and LDH activities, increased ALP activity, 65% increase in liver weight)		
			Renal	60 M			
19	Rat (NS)	360 d ad libitum (F)	Hepatic	0.4 M	2 M (increased liver weight)	20 M (focal necrosis, enlargement of hepatocytes, nuclear pyknosis, vacuolation, margination)	Dikshith et al. 1991a technical
			Renal	2 M		20 M (tubular necrosis, glomerular degeneration)	
20	Rat (NS)	90 d 1x/d (GO)	Hepatic		5 M (decreased liver and serum GOT and alkaline phosphatase activities)		Dikshith et al. 1991b technical
21	Rat (Charles Foster)	180 d 1x/d (GO)	Bd Wt		3 M (17% decrease in body weight gain)		Gautam et al. 1989 technical

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
22	Rat (CFT-Wistar)	7 wk ad libitum (F)	Hepatic		90M (decreased hepatic vitamin A content, GOT, GPT ALP, and beta-GLR activities, 121% increase in liver weight)		Joseph et al. 1992b technical
			Bd Wt		90M (17% decrease in body weight gain)		
23	Rat (CFT-Wistar)	7 wk ad libitum (F)	Hemato		90M (decreased white blood cell counts)		Joseph et al. 1992c technical
24	Rat (NS)	30 d 1x/d (GO)	Hepatic	50 M			Khanna et al. 1990 technical
			Renal	50 M			
25	Rat (Wistar)	90 d ad libitum (F)	Bd Wt		20F (significantly decreased body weight gain)		Nagaraja and Desiraju 1994 technical
26	Rat (Wistar)	13 wk ad libitum (F)	Hemato	4.5 M 5 F	22.5 M (decreased red blood 25 F cell, leukocyte, and hemoglobin concentrations)		Van Velsen et al. 1986 beta
			Hepatic		0.18[c] M (hyalinization of 0.2 F centrilobular cells)	4.5 M (hyalinization of 5 F centrilobular cells, focal cell necrosis, increased mitoses)	
			Renal	4.5 M	22.5 M (calcinosis in males)		
			Bd Wt	4.5 M 5 F	22.5 M (15% decrease in body 25 F weight)		
27	Mouse (dd)	32 wk ad libitum (F)	Hepatic	18 M 20 F	54 M (nuclear irregularities in 60 F foci of enlarged hepatocytes)		Hanada et al. 1973 beta

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference/ Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
28	Mouse (dd)	24 wk ad libitum (F)	Hepatic		18M (centrilobular hypertrophy)		Ito et al. 1973 alpha
29	Mouse (dd)	24 wk ad libitum (F)	Hepatic		45M (centrilobular hypertrophy)		Ito et al. 1973 beta
30	Mouse (dd)	24 wk ad libitum (F)	Hepatic		90M (centrilobular hypertrophy)		Ito et al. 1973 delta
31	Mouse (Swiss)	2-8 mo ad libitum (F)	Hepatic		90 (100% increase in liver weight, decreased G6P and FDP activity, glycogen accumulation, smooth endoplasmic reticulum proliferation)		Karnik et al. 1981 technical
32	Mouse (HPB)	50 wk ad libitum (F)	Hepatic		90M (hyperplastic nodules)		Tryphonas and Iverson 1983 alpha
Immunological/Lymphoreticular							
33	Rat (Wistar)	13 wk ad libitum (F)				22.5 M (cortical atrophy in thymus) 25 F	Van Velsen et al. 1986 beta
34	Mouse (B6C3F1)	30 d ad libitum (F)		20 F	60F (decr. lymphoproliferative responses to T-cell mitogens, decr. natural killer cytolytic activity)		Cornacoff et al. 1988 beta

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
Neurological							
35	Rat (NS)	3 mo 6 d/wk 1x/d (GO)			50M (increased dopamine and decreased serotonin, acetylcholine, norepinephrine in cerebral cortex, behavioral changes, increased brain wave frequency)		Anand et al. 1991 technical
36	Rat (NS)	360 d 1x/d (F)		0.04 M		0.4 M (convulsions, tremors, hindlimb paralysis, salivation)	Dikshith et al. 1991a technical
37	Rat (NS)	120 d 1x/d (GO)			50M (increased motor activity, decreased resting stereotypic time)		Gopal et al. 1992 technical
38	Rat (Wistar)	30 d ad libitum (F)		106.2 M			Muller et al. 1981 alpha
39	Rat (Wistar)	30 d ad libitum (F)			66.3 M (reduced tail nerve conduction velocity)		Muller et al. 1981 beta
40	Rat (Wistar)	90 d ad libitum (F)			20F (increased GABA levels, increased GAD activity, decreased glutamate levels)		Nagaraja and Desiraju 1994 technical
41	Rat (Wistar)	13 wk ad libitum (F)		4.5 M 5 F		22.5 M (ataxia, coma) 25 F	Van Velsen et al. 1986 beta

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
Reproductive							
42	Rat (NS)	360 d 1x/d (F)		2 M		20 M (testicular degeneration)	Dikshith et al. 1991a technical
43	Rat (Charles Foster)	180 d 1x/d (GO)			3M (6% decrease in vas deferens weight, degeneration of inner muscle and cell layers)		Gautam et al. 1989 technical
44	Rat (CFT-Wistar)	7 wk ad libitum (F)				90 M (decreased testes, epididymides, and seminal vesicle weights, 30% decrease in sperm count)	Pius et al. 1990 technical
45	Rat (Charles Foster)	180 d 1x/d (GO)			3M (decreased seminiferous tubular and Leydig cell nuclear diameter)	6 M (seminiferous tubular degeneration)	Roy Chowdhury and Gautam 1990 technical
46	Rat (Wistar)	13 wk ad libitum (F)		0.9 M 0.2 F	4.5 M (decreased testes 1.0 F weight) (increased ovary weights)	22.5 M (atrophy of ovary and 25 F testes, hyperplastic and vacuolized endometrium epithelium in uterus)	Van Velsen et al. 1986 beta
47	Mouse (B6C3F1)	30 d ad libitum (F)		60 F			Cornacoff et al. 1988 beta
48	Mouse (Swiss)	3 mo ad libitum (F)				90 M (increased testis weight, degeneration of seminiferous tubules, decreased spermatocytes)	Nigam et al. 1979 technical

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
Developmental							
49	Rat (Wistar)	60 d ad libitum (G)			10F (alterations in levels of dopamine, serotonin, and noradrenaline in pup brains)		Nagaraja and Desiraju 1994 technical
50	Rat (Wistar)	21GD, 28 LD, 28 LD (F)			5 (increased liver weight in pups exposed during gestation and lactation)	20 (increased pup mortaility)	Srinivasan et al. 1991a beta
Cancer							
51	Rat (Wistar)	20 wk ad libitum (F)				2 F (CEL: increase in preneoplastic hepatic foci)	Schroter et al. 1987 alpha
52	Rat (Wistar)	20 wk ad libitum (F)				3 F (CEL: increase in preneoplastic hepatic foci)	Schroter et al. 1987 beta
53	Mouse (dd)	32 wk ad libitum (F)				18 M (CEL: hepatoma) 60 F	Hanada et al. 1973 alpha
54	Mouse (dd)	24 wk ad libitum (F)				45 M (CEL: hepatocellular carcinoma)	Ito et al. 1973 alpha
55	Mouse (DDY)	16-36 wk ad libitum (F)				90 M (CEL: hepatocellular carcinoma)	Ito et al. 1976 alpha
56	Mouse (Swiss)	2-4 mo ad libitum (F)	Hepatic			90 (CEL: liver tumors)	Karnik et al. 1981 technical

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
57	Mouse (DDY, ICR, DBA/2, C57BL/6, C3H/He)	24 wk ad libitum (F)				90 M (CEL: hepatocellular carcinoma) 100 F	Nagasaki et al. 1975b alpha
58	Mouse (Swiss)	2-8 mo ad libitum (F)				90 (CEL: hepatocellular carcinoma)	Thakore et al. 1981 technical
59	Mouse (HPB)	50 wk ad libitum (F)				90 M (CEL: hyperplastic nodules and adenomas in liver)	Tryphonas and Iverson 1983 alpha
60	Mouse (DD)	16-36 wk ad libitum (F)				90 M (CEL: hepatoma)	Tsukada et al. 1979 alpha
CHRONIC EXPOSURE							
Systemic							
61	Rat (Wistar)	107 weeks ad libitum (F)	Hepatic	0.7 M 0.8 d F	3.5 M (focal necrosis, fatty 4 F degeneration, 32% increase in liver weight)		Fitzhugh et al. 1950 alpha
			Renal	7 M 8 F	56 M (focal nephritis) 64 F		
			Bd Wt	7 M	56 M (18% decrease in body weight gain)		
				8 F	64 F (13% decrease in body weight gain)		

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
62	Rat (Wistar)	107 weeks ad libitum (F)	Hepatic		0.7 M (focal necrosis, fatty 0.8 F degeneration, 33% increase in liver weight)		Fitzhugh et al. 1950 beta
			Renal	7 M 8 F	56 M (focal nephritis) 64 F		
			Bd Wt	56 M 0.8 F	8F (12% decrease in body weight gain)		
63	Rat (Wistar)	107 weeks ad libitum (F)	Hepatic	0.7 M 0.8 F	3.5 M (very slight microscopic 4 F damage)	7 M (focal necrosis, fatty 8 F degeneration, 36% increase in liver weight)	Fitzhugh et al. 1950 technical
			Renal	7 M 8 F	56 M (focal nephritis) 64 F		
			Bd Wt	7 M 8 F	56 M (decreased body weight 64 F gain)		
Neurological							
64	Mouse (Swiss)	80 wk ad libitum (F)				17 (convulsions)	Kashyap et al. 1979 technical
65	Mouse (Swiss)	80 wk 1x/d (GO)				10 (convulsions)	Kashyap et al. 1979 technical
Cancer							
66	Rat	72 wk ad libitum (F)				50 (CEL: hepatocellular carcinoma)	Ito et al. 1975 alpha
67	Mouse (Swiss)	80 wk 1x/d (GO)				10 (CEL: hepatocellular carcinoma)	Kashyap et al. 1979 technical

TABLE 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference/ Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)	
68	Mouse (Swiss)	80 wk ad libitum (F)					17 (CEL: hepatocellular carcinoma)	Kashyap et al. 1979 technical
69	Mouse (Swiss)	20 mo ad libitum (F)					21.3 M (CEL: hepatocellular carcinoma)	Munir et al. 1983 technical
70	Mouse (CF1)	104 wk ad libitum (F)					34 (CEL:hepatocellular carcinoma)	Thorpe and Walker 1973 beta

[a]The number corresponds to entries in Figure 2-3.
[b]Used to derive an acute-duration oral Minimal Risk Level (MRL) of 0.2 mg/kg/day for beta-HCH; 19 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability) = 0.19 mg/kg/day.
[c]Used to derive an intermediate-duration oral Minimal Risk Level (MRL) of 0.0006 mg/kg/day for beta-HCH; 0.18 mg/kg/day divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, 10 for human variability) = 0.0006 mg/kg/day.
[d]Used to derive a chronic-duration oral Minimum Risk Level (MRL) of 0.008 mg/kg/day for alpha-HCH; 0.8 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability) = 0.008 mg/kg/day.

ALP = alkaline phosphatase; Bd Wt = body weight; CEL = cancer effect level; d = day(s); F = female; FDP = fructose-1,6-diphosphatase; GABA = gamma-aminobutyric acid; GAD = glutamate decarboxylase; GLR = glucuronidase; GOT = glutamate oxaloacetate transaminase; G6P = glucose-6-phosphatase; GPT = glutamate pyruvate transaminase; Hemato = hematological; LD50 = lethal dose, 50% kill; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolism; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s).

Figure 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral

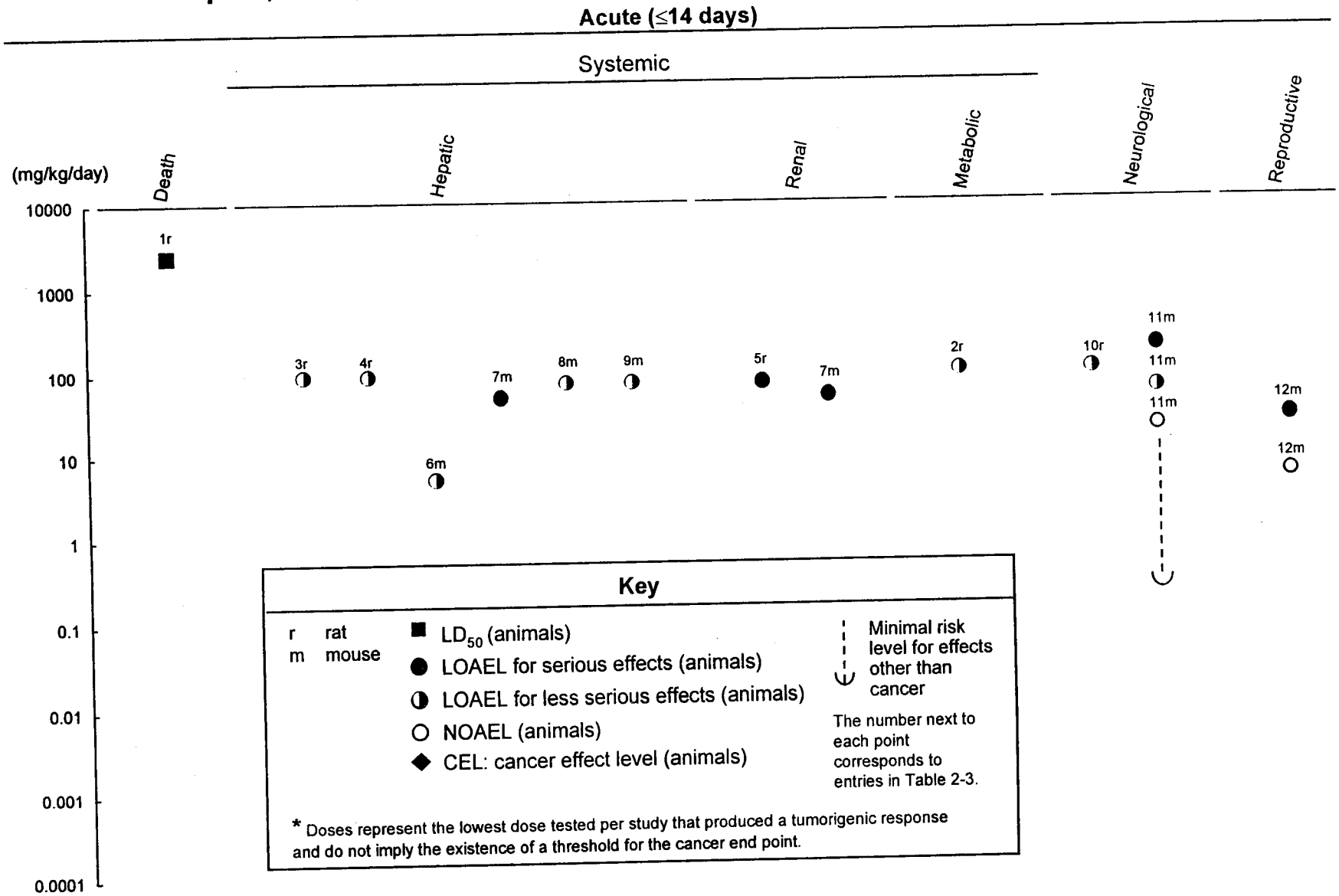


Figure 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (cont.)

Intermediate (15-364 days)

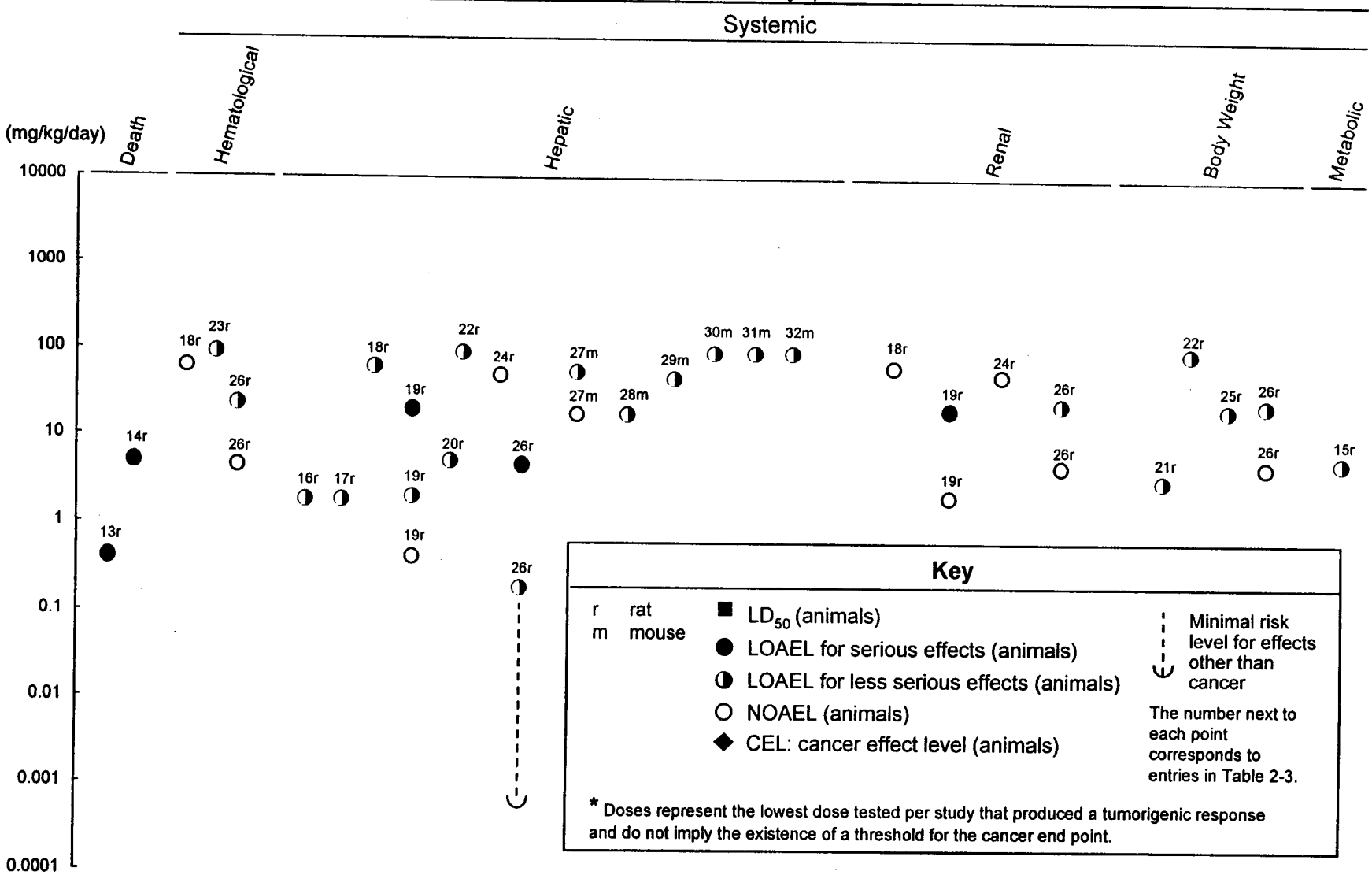


Figure 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (cont.)

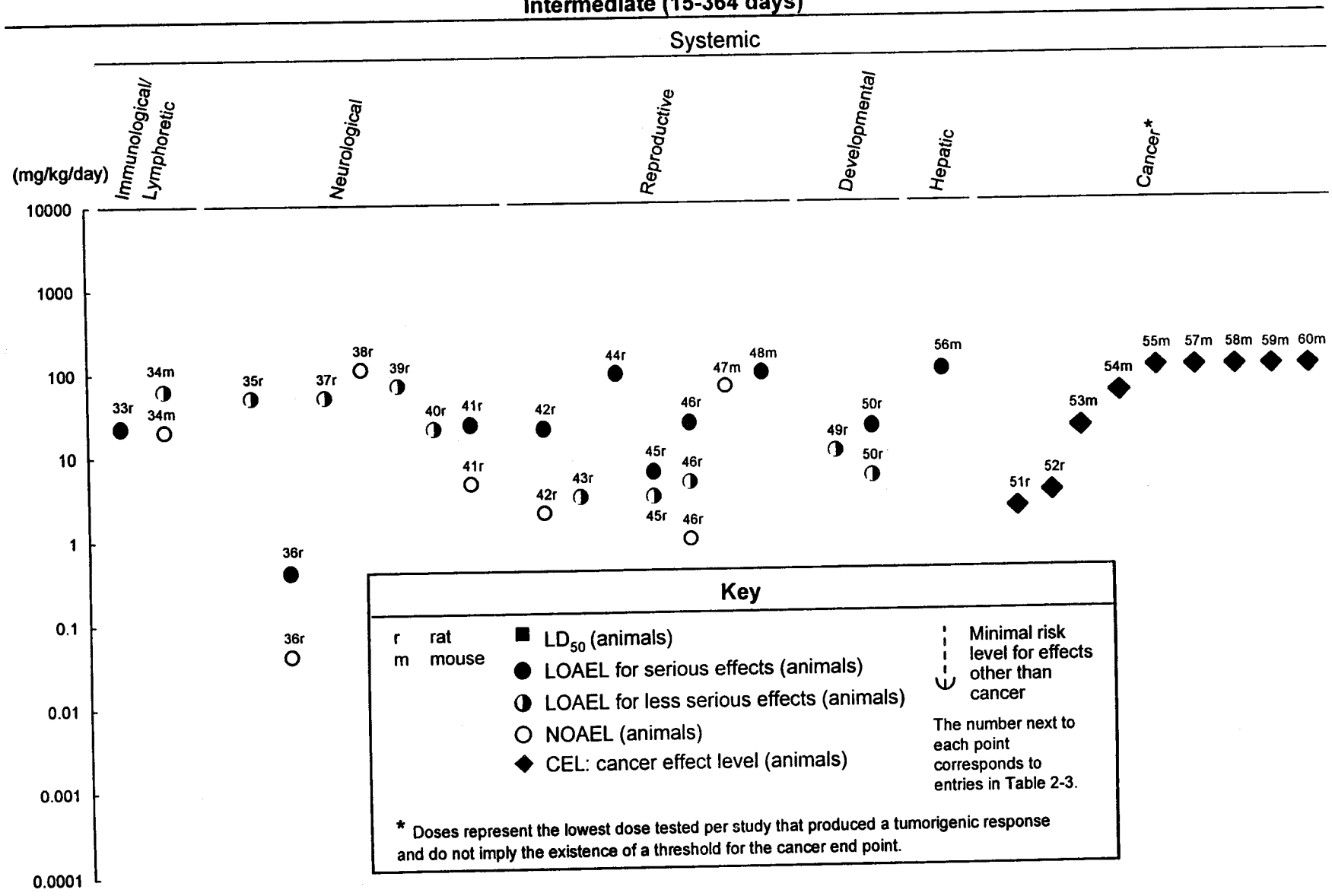
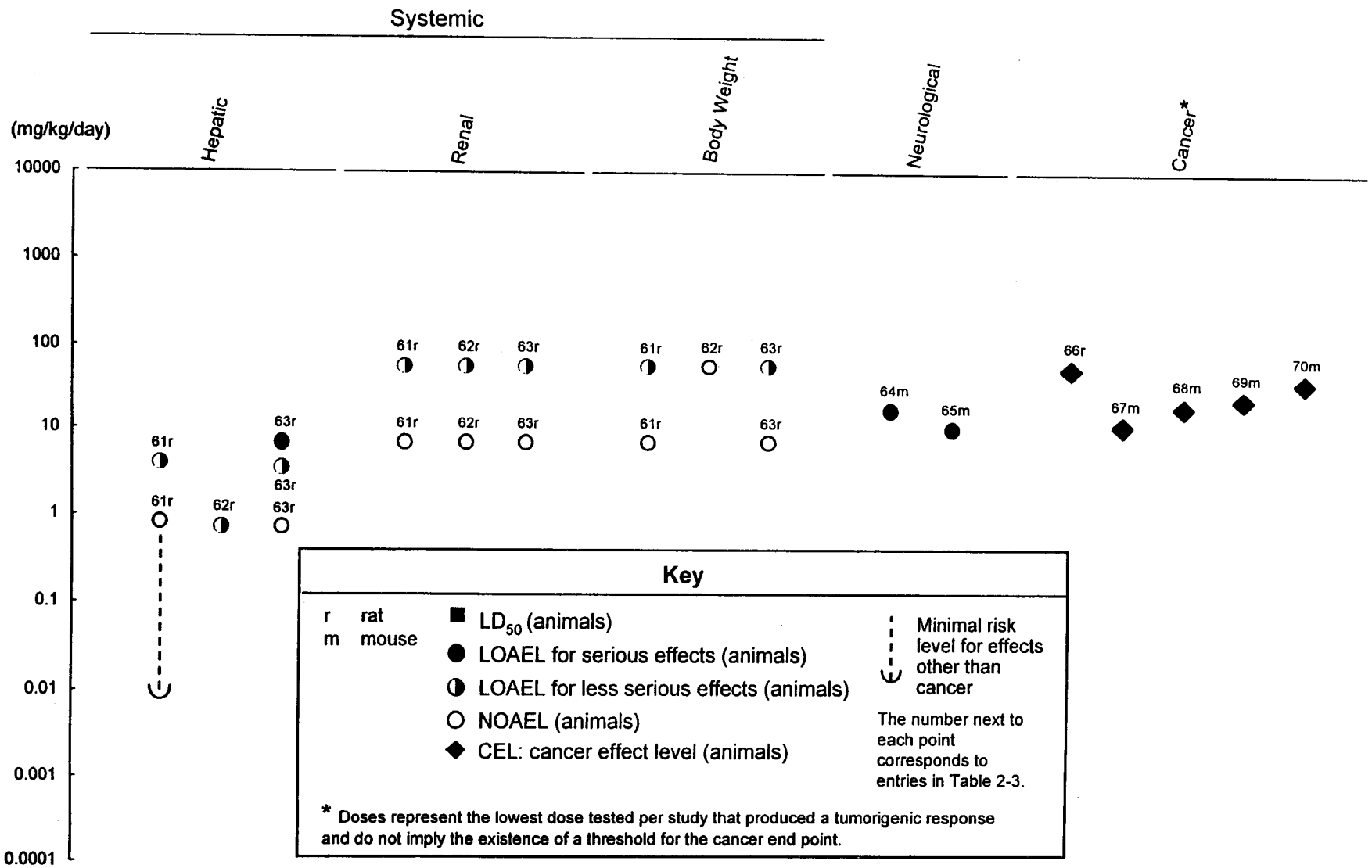


Figure 2-3. Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Technical-Grade Hexachlorocyclohexane - Oral (cont.)

Chronic (≥365 days)



2.2.2.2 Systemic Effects

No studies were located regarding respiratory, dermal, or ocular effects in humans or animals following oral exposure to HCH. The animal studies in which systemic effects of HCH were examined, in most cases, used isomers of >99% purity. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-2 and 2-3.

Cardiovascular Effects. There are no reports of cardiovascular damage from γ-HCH or any other HCH isomer.

Gastrointestinal Effects. Decreased appetite, vomiting, nausea, and diarrhea have been observed in humans following ingestion of γ-HCH in contaminated food; exposure levels were not reported, but exposure was inferred from levels of γ-HCH measured in urine (Nantel et al. 1977). Vomiting and nausea are usual manifestations of lindane ingestion (Sunder Ram Rao et al. 1988).

The activities of the digestive enzyme maltase on brush border membrane of rat jejunum are reported to be inhibited by oral treatment with 20 mg/kg γ-HCH over 7 and 15 days (Moreno et al. 1996). In addition, γ-HCH has been shown to have an effect on intestinal functions such as uptake of glucose, glycine, and calcium in rats (Labana et al. 1997), and the effect depends on the nutritional status of the animals.

Hematological Effects. A woman who committed suicide by drinking γ-HCH was found to have disseminated intravascular coagulation during the period when serum γ-HCH levels were elevated (Sunder Ram Rao et al. 1988). No other reports were found on the possible effect of γ-HCH on blood-clotting factors in humans.

No hematological effects were noted in beagle dogs exposed to 12.5 mg γ-HCH/kg/day in the diet for 32 weeks or to 2.9 mg γ-HCH/kg/day in the diet for 104 weeks (Rivett et al. 1978). Twelve-week studies in rats, using lower doses (10 mg/kg/day), support this finding (Suter 1983). However, exposure to 22.5 mg β-HCH/kg/day in the diet for 13 weeks in rats was found to be more toxic, resulting in a statistically significant decrease in numbers of red blood cells and white blood cells and reduced hemoglobin and packed cell volume values (Van Velsen et al. 1986). Significant decreases in total white blood cell counts and

clotting time were reported in rats fed vitamin A-free diets containing technical-grade HCH at a dose level of 90 mg/kg/day for 7 weeks (Joseph et al. 1992c). In rats fed a vitamin A-supplemented diet containing the same dose level of technical-grade HCH, a significant reduction in total white blood cell count, but not red blood cell count, was observed (Joseph et al. 1992c). Significant suppression in bone marrow cellularity, erythrocyte precursors, and granulocyte-macrophage progenitor cells, and residual progenitor cell damage were reported in male $B6C3F_1$ mice given 20 or 40 mg γ-HCH/kg/day by gavage in corn oil for 3 days (Hong and Boorman 1993). Following 10 days of exposure to 10 or 20 mg γ-HCH/kg/day, dose-dependent decreases in bone marrow cellularity, granulocyte-macrophage progenitor cells, and pluripotent bone marrow stem cells were noted (Hong and Boorman 1993).

No hematological effects were seen in rats following oral exposure to 60 mg/kg/day technical-grade HCH for 30 days (Dikshith et al. 1989a).

Musculoskeletal Effects. In humans, ingestion of a single dose of approximately 15–30 mL γ-HCH powder was associated with seizures and limb muscle weakness and necrosis (Munk and Nantel 1977); a muscle biopsy was conducted on day 15 after ingestion and showed no evidence of denervation or neuropathy. Widespread striatal muscle necrosis was seen in a woman who died 11 days after intentionally ingesting 8 ounces of a 20% lindane solution (Sunder Ram Rao et al. 1988).

Decreased cross-sectional bone area was found in young rats treated with 20 mg/kg/day of γ-HCH by gavage for 10 weeks (Andrews and Gray 1990). Myelotoxicity, manifested as significant, dose-dependent decrease in marrow progenitor numbers, was seen in mice exposed to 10 or 20 mg/kg/day lindane for 10 days (Hong and Boorman 1993).

Hepatic Effects. No studies were located regarding hepatic effects in humans following oral exposure to HCH.

Significantly increased liver microsomal 7-ethoxycoumarin-o-dealkylase activity was found in Osborne-Mendel rats exposed to 11.2 mg γ-HCH/kg/day and in CF_1 and $B6C3F_1$ strain mice exposed to 23.6 and 50.5 mg/kg/day in the diet for 3 days (Oesch et al. 1982). No adverse effects were noted in rats exposed to 10 mg/kg/day for a minimum of 4 days (Joy et al. 1982). No significant increase in liver weight was reported, but no histopathological examinations were performed to confirm the presence or absence of toxicity. Hepatocellular damage as indicated by elevation in serum aminotransferases and decrease in hepatic

soluble enzymes was found in rats given 72 mg/kg/day γ-HCH for 2 weeks (Srinivasan and Radhakrishnamurty 1988). Significant increases in hepatic microsomal cytochrome P-450 levels and increases in hepatic microsomal superoxide anion production and cytoplasmic superoxide dismutase activity and lipid peroxidation were found in Wistar rats fed diets containing 1.8 mg/kg/day γ-HCH for 15 or 30 days (Barros et al. 1991). Male Wistar rats fed 13.5 mg lindane/kg/day in their diet for 12 days exhibited decreased activities of liver lipogenic enzymes and increased levels of serum triglycerides (Boll et al. 1995). Focal degeneration of hepatocytes was noted in rabbits given γ-HCH at a dose of 7 mg/kg/day by gavage for 4 weeks (Grabarczyk et al. 1990; Kopec-Szlezak et al. 1989). Rabbits treated with 4.21 mg lindane/kg/day by gavage for 28 days exhibited a significant increase of plasma alkaline phosphatase and alanine aminotransferase activities immediately following initiation of dosing; these activities returned to control levels by day 14 (Cerón et al. 1995). Activity of aspartate aminotransferase also increased immediately following dosing and remained elevated up to 7 days postexposure (day 35). Lindane residues were detected in the blood.

Exposure for 3 months (12 weeks) resulted in increases in liver microsomal mixed-function oxidase activity in rats and mice and a significant increase in absolute and relative liver weights in female rats fed 10.6 and 32.3 mg/kg/day and male and female CF_1 mice fed 21.1 mg/kg/day; histopathological examinations were not performed (Oesch et al. 1982). Liver centrilobular hypertrophy increased in a dose-dependent manner beginning at a dose of 0.4 mg lindane/kg/day in Wistar rats exposed in their diet for 12 weeks (Suter 1983). Liver cell lipospheres were reported in rats fed 2.5 mg γ-HCH/kg/day in the diet for 32 weeks (Ortega et al. 1957). In mice, administration of 90 mg γ-HCH/kg/day in the diet for 24 weeks was reported to result in centrilobular hypertrophy (Ito et al. 1973). Hanada et al. (1973) reported liver cancer in mice fed 78 mg/kg/day in the diet for 32 weeks. Other studies of intermediate-duration exposure (3–48 weeks) have reported slight liver effects or increased liver weight in mice exposed to 18 mg/kg/day of α-HCH, 45 mg/kg/day of β-HCH, and 90 mg/kg/day for δ-HCH and γ-HCH. (Ito et al. 1975). These studies were limited by either a small sample size or lack of statistical analysis.

Chronic exposure of rats to 7–8 mg/kg/day γ-HCH in the diet for 38–70 weeks was reported to result in liver necrosis and fatty degeneration (Fitzhugh et al. 1950). A dose-related increase in periacinal hepatocytic hypertrophy was seen in Wistar rats given 7–8 mg lindane/kg/day in the diet for 104 weeks (Amyes 1990). No liver effects were reported in dogs exposed to 2.9 mg/kg/day for 104 weeks (Rivett et al. 1978). In mice, chronic administration of 13.6–27.2 mg γ-HCH/kg/day in the diet was associated with an increased incidence of liver cancer (NCI 1977; Wolff et al. 1987) (see Section 2.2.2.8).

Similar liver effects were reported in animals following intermediate- or chronic-duration exposure to α-HCH in the diet. Administration of 1.8 mg/kg/day α-HCH in the diet to rats for 15 or 30 days resulted in increases in hepatic cytochrome P-450 content, hepatic lipid peroxidation, and hepatic microsomal superoxide production (Barros et al. 1991). Ito et al. (1975) reported liver cell hypertrophy and hyperplasia in rats exposed to 45 mg/kg/day α-HCH for 24–48 weeks. Hypertrophied liver cells were reported in mice fed 18 mg/kg/day α-HCH and 45 mg/kg/day β-HCH for 24 weeks (Ito et al. 1973), and hepatomegaly was reported in mice exposed to 90 mg/kg/day in the diet for 50 weeks (Tryphonas and Iverson 1983). Liver cancer has also been reported in mice given 18–90 mg α-HCH/kg/day for 16–36 weeks (Hanada et al. 1973; Ito et al. 1973, 1976; Nagasaki et al. 1975; Tsukada et al. 1979) (see Section 2.2.2.8). Long-term exposure to lower doses of α-HCH was reported to result in fatty degeneration and focal necrosis in rats exposed to 3.5–4.0 mg/kg/day for 36–56 weeks (Fitzhugh et al. 1950), and liver cancer was reported in rats administered 50 mg/kg/day in the diet for 72 weeks (Ito et al. 1975).

Significant increases in liver weight and in the levels of hepatic cytochrome P-450, triglycerides, phospholipids, and cholesterol were observed in rats administered 90 mg/kg/day β-HCH in the diet for 2 weeks (Ikegami et al. 1991a, 1991b); decreases in cytochrome c reductase activity were also reported. Intermediate and chronic exposure to β-HCH in the diet is also associated with liver effects in animals. A dose-dependent increase in liver weight was noted in rats exposed for 13 weeks to 0.18–4.5 mg β-HCH/kg/day; the increase was significant at doses of >1 mg/kg/day (Van Velsen et al. 1986). Liver cell hypertrophy was reported in rats fed 25 or 50 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975). In mice, exposure to 45 mg/kg/day for 24 weeks resulted in liver cell hypertrophy (Ito et al. 1973), and exposure to 54–57 mg/kg/day for 32 weeks resulted in hepatic foci of degeneration (Hanada et al. 1973). β-HCH was not found to be carcinogenic in rats or mice exposed for 24–48 weeks (Hanada et al. 1973; Ito et al. 1975). Chronic exposure to lower doses of β-HCH resulted in fatty degeneration and necrosis in the liver of mice fed 3.5–4 mg/kg/day for 36–56 weeks (Fitzhugh et al. 1950), and Thorpe and Walker (1973) reported liver cancer in mice fed 34 mg/kg/day for 26 months.

Liver hypertrophy was observed in rats fed with 45 mg/kg/day of α-, β-, or δ-HCH in the diet for 24 or 48 weeks (Ito et al. 1975) and in mice fed 18 mg/kg/day α-HCH in the diet for 24 weeks (Ito et al. 1973). The toxicity of ingested δ-HCH has not been investigated following chronic exposure.

Technical-grade HCH was reported to cause increases in liver weight and enzymatic activity (e.g., alkaline phosphatase, aminotransferases) in male Swiss mice given 72 mg/kg in the diet for 2 weeks (Ravinder et al.

1989). The same dosing regime also caused significantly increased serum triglycerides, phospholipids, and cholesterol, as well as hypertrophy of hepatocytes with enlargement of nuclei, centrilobular degeneration, and focal necrosis (Ravinder et al. 1990). Statistically significant decreases in the liver activity of glutamic oxaloacetate transaminase (GOT) and lactate dehydrogenase (LD) were observed in pregnant mice administered a single dose of technical-grade HCH (5 mg/kg) on gestation day 9 (Dikshith et al. 1990). Pregnant mice dosed with 25 mg/kg technical-grade HCH experienced a statistically significant decrease in glutamic pyruvic transaminase(GPT) and alkaline phosphatase (AP) activity. Virgin mice administered a single dose of 5–200 mg/kg technical-grade HCH had statistically significant decreases in liver activity of GOT and GPT. Statistically significant increases in liver AP activity were observed in the virgin mice administered 25–200 mg/kg technical-grade HCH. However, with the exception of GOT activity in pregnant mice, the dose response relationships were questionable (Dikshith et al. 1990). There were also no corresponding pathological changes in the liver. Similar effects were seen in male, but not female, rats given 5 or 25 mg/kg/day by gavage for 90 days (Dikshith et al. 1991b). A 65% decrease in liver weight, decreased liver aspartate aminotransferase and lactate dehydrogenase activities, and increased alkaline phosphatase activity were noted in male rats given 60 mg/kg by gavage for 30 days, but animals had normal liver histology (Dikshith et al. 1989a). However, enlargement of hepatocytes, nuclear pyknosis, margination, and vacuolation were observed in rats fed 20 mg/kg/day technical-grade HCH in the diet for 360 days (Dikshith et al. 1991a). No adverse hepatic effects were seen in rats treated with 50 mg/kg/day technical-grade HCH for 30 days (Khanna et al. 1990).

Technical-grade HCH was reported to deplete the hepatic vitamin A content, decrease enzyme activities, and increase liver weight in male rats fed a vitamin A-free diet containing 90 mg/kg/day HCH for 7 weeks (Joseph et al. 1992b). Fatty degeneration and necrosis of the liver were found in rats exposed to 7–8 mg/kg/day of technical-grade HCH for 33–61 weeks (Fitzhugh et al. 1950); these effects were more pronounced at 56–64 mg/kg/day. Mice treated daily with 50 mg/kg/day technical-grade HCH for 1, 5, or 15 days by oil gavage exhibited congestion of hepatic portal vessels and central vein, swollen hepatic cells with vacuolar or parenchymatous degeneration, and fatty changes in periportal and centrilobular cells (Philip et al. 1989). Mice fed diets containing 90 mg/kg/day of HCH for 8 months exhibited increased liver weight, glycogen accumulation, and decreased glucose-6-phosphatase and fructose-1,6-diphosphatase activities (Karnik et al. 1981). Technical-grade HCH was also reported to cause liver cancer in mice following exposure to 90 mg/kg/day in the diet for 2–8 months (Karnik et al. 1981; Thakore et al. 1981) or exposure to 10–50 mg/kg/day for 80–88 weeks (Kashyap et al. 1979; Munir et al. 1983) (see Section 2.2.2.8).

Based on the occurrence of hepatic effects in rats and mice exposed to β-HCH, an intermediate MRL of 0.0006 mg/kg/day has been calculated from the LOAEL of 0.18 mg β-HCH/kg/day (Van Velsen et al. 1986), as described in the footnote in Table 2-3.

An MRL of 0.01 mg/kg/day has been derived for intermediate-duration oral exposure to α-HCH, based on a NOAEL of 1.0 mg/kg/day for hepatic effects in male and female rats (Fitzhugh et al. 1950).

Renal Effects. Progressive renal failure was seen in a woman who died 11 days after intentionally ingesting 8 ounces of a 20% lindane solution (Sunder Ram Rao et al. 1988). The myoglobin release resulting from muscle lysis in this case led to kidney shutdown which was the ultimate cause of death.

Male Fischer-344 rats receiving gavage doses of 10 mg/kg/day of γ-HCH for 4 days showed α-2μ-globulin staining in the kidney cortex. Histopathological changes in the proximal tubule epithelial cells included accumulation of protein droplets, hypertrophy and necrosis, pyknotic nuclei, cellular exfoliation, and regenerative epithelium (Dietrich and Swenberg 1990, 1991). These effects did not occur or were seen to a very slight extent in Fischer-344 male controls, Fischer-344 female exposed rats, or exposed NBR rats (a strain that does not synthesize α-2μ-globulin). These results indicate that damage to male rat kidneys by γ-HCH may be caused by α-2μ-globulin, a protein that is not present in humans. Thus, it is unlikely that humans are at risk for developing this type of pathology from γ-HCH (EPA 1991a). Other biochemical changes indicative of kidney injury, such as significantly increased excretion of glucose in urine, and histological changes, such as hypertrophy and degeneration of the renal tubular epithelia, were observed in Wistar rats exposed to 72 mg/kg/day of γ-HCH for up to 2 weeks (Srinivasan and Radhakrishnamurty 1988; Srinivasan et al. 1984).

However, no renal effects other than significantly increased kidney weight were observed in rats exposed to up to 5–50 mg γ-HCH/kg/day in the diet for up to 40 days (Desi 1974); histological examination of the kidney did not reveal any changes. Slight kidney damage (calcified tubular casts) was reported in rats exposed to 9–10 mg γ-HCH/kg/day for an average of 39.7 weeks (Fitzhugh et al. 1950); the results of this study are limited by poor survival in control and treated animals at all doses. Male rats exposed for 2 years to lindane in their diet exhibited hyaline droplets in the renal proximal tubules at 0.07 mg/kg/day, and pale kidneys, increased kidney weights and urine volumes, and higher urinary protein excretions and tubular necrosis at 7 mg/kg/day (Amyes 1990). Hyaline droplet formation also occurred in a dose-dependent manner in rats treated with 0.02–10 mg lindane/kg/day in their diets for 12 weeks (Suter 1983). Dose-dependent

incidents of renal tubular distension and degeneration were seen in this study beginning at a dose of 2 mg lindane/kg/day.

Fitzhugh et al. (1950) reported kidney damage (nephritis and basal vacuolation) in rats fed 72–80 mg α-HCH/kg/day for an average of 35.9 weeks; no such effects were observed in rats fed 5 mg/kg/day. Poor survival was noted in both control and treated animals.

Renal effects have also been noted in rats exposed to β-HCH in the diet. Srinivasan et al. (1984) reported significantly increased excretion of glucose in urine and increased excretion of creatinine and urea as well as hypertrophy and degeneration of the renal tubular epithelia in rats exposed to 72 mg β-HCH/kg/day for up to 2 weeks. Van Velsen et al. (1986) reported significantly increased kidney weights in female rats exposed to 0.18 mg β-HCH/kg/day for 13 weeks; males did not show a significant increase until they were exposed to a dose of 4.5 mg/kg/day. At 22.5 mg/kg/day, both males and females exhibited renal calcinosis in the outer medulla; however, the female controls also exhibited calcinosis. The study authors noted that renal calcinosis is common in female rats but that this finding was of significance in males (Van Velsen et al. 1986). Fitzhugh et al. (1950) also examined the renal effects of exposure to β-HCH in rats that died after an average of 4.4 weeks and found nephritis and basal vacuolation similar to that described in rats exposed to α-HCH; poor survival due to unspecified causes was reported in both control and treated animals.

Nephritis, pigmentation, and basal vacuolation were also observed in rats fed 56–64 mg technical-grade HCH/kg/day (64% α-HCH, 10% β-HCH, 13% γ-HCH, 9% δ-HCH, and 1.3% ε-HCH) in the diet for an average of 32.9–64.6 weeks (Fitzhugh et al. 1950); poor survival (for which there was no explanation) was noted in both control and treated animals. Tubular necrosis and glomerular degeneration was seen in animals exposed for 360 days to 20 mg/kg/day of technical-grade HCH (Dikshith et al. 1991a), but no renal effects were seen in rats exposed to 60 mg/kg/day technical-grade HCH for 30 days by oil gavage (Dikshith et al. 1989a). Mice treated daily with 50 mg/kg/day technical-grade HCH for 1, 5, or 15 days by oil gavage exhibited congestion of blood vessels and glomerular tufts, swollen tubules with hyaline casts, cystic dilation, fatty changes, some interstitial hemorrhaging in the medulla, and epithelial cell vacuolation (Philip et al. 1989). No adverse effects were seen in rats treated with 50 mg/kg/day technical-grade HCH for 30 days (Khanna et al. 1990).

Endocrine Effects. No studies were located regarding endocrine effects in humans or animals following oral exposure to HCH.

Body Weight Effects. No studies were located regarding body weight effects in humans following oral exposure to HCH.

Significantly decreased body weight gain has been seen in rats treated orally with 800 ppm α- (Fitzhugh et al. 1950), 250 mg/kg feed β- (Fitzhugh et al. 1950; Van Velsen et al. 1986), 40 mg/kg/day γ- (Fitzhugh et al. 1950; Laws et al. 1994), and 10 or 20 mg/kg/day technical-grade HCH (Gautam et al. 1989; Joseph et al. 1992b; Nagaraja and Desiraju 1994).

Metabolic Effects. No studies were located regarding metabolic effects in humans following oral exposure to HCH.

Increased phosphoinositide turnover and generation of second messengers from phosphoinositides were seen in erythrocyte membranes from female rats treated by gavage with a single dose of 100 mg/kg technical-grade HCH, or with doses of 5 mg/kg/day technical-grade HCH for 3–6 months, 5 days/week (Agrawal et al. 1995). The latter exposure regime also resulted in a significant decrease in phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate in erythrocyte membrane and cerebrum; the levels decreased with increased time of treatment (3–6 months).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans following oral exposure to HCH.

Some evidence of possible immunotoxic effects of γ-HCH is available from animal studies. Immuno-suppression, as measured by decreased agglutinin titers against typhoid vaccine and *Salmonella* vaccine, was reported in rats exposed by gavage to 6.25 and 25 mg γ-HCH/kg/day for 5 weeks (Dewan et al. 1980) and in rabbits exposed by capsules 5 times each week to 1.5, 6, and 12 mg/kg/day for 5–6 weeks (Desi et al. 1978). Dose related decreases in thymus and spleen weights were observed in mice gavaged with 10–20 mg/kg/day γ-HCH for 10 days and decreased thymus weight was observed in mice gavaged with 20–40 mg/kg/day γ-HCH for 3 days (Hong and Boorman 1993). The primary antibody response to sheep red blood cells was

suppressed in albino mice after exposure to 9 mg/kg/day γ-HCH in their diet for 12 weeks (Banerjee et al. 1996). Suppression of secondary antibody response was also observed after 3 weeks exposure to 9 mg/kg/day γ-HCH and after 12 weeks of 5.4 mg/kg/day lindane exposure. Decreased lymphoproliferative responses to mitogens were seen in mice exposed to 60 mg/kg/day β-HCH in the diet for 30 days (Cornacoff et al. 1988). There were no associated changes in immunoglobulins, red blood cell counts, or histology of the thymus, spleen, or lymph nodes. Cortical atrophy of the thymus was observed in rats fed 22.5–25 mg/kg/day β-HCH (Van Velsen et al. 1986). A biphasic dose-dependent immunological effect of γ-HCH on components of cell- and humoral-mediated immunity, characterized by initial stimulation followed by immunosuppression, was reported in mice fed 0.012, 0.12, or 1.2 mg γ-HCH/kg/day for 24 weeks (Meera et al. 1992). In addition, histological examinations revealed decreased lymphocyte populations in the thymus and lymph nodes and a reduction in overall cellularity in the spleen and necrosis of the thymus at 1.2 mg/kg/day. The LOAEL values for immunological effects are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-2 and 2-3.

Based on immunological effects of γ-HCH on components of cell- and humoral-mediated immunity in mice, an intermediate MRL of 1×10^{-5} mg/kg/day has been calculated from the LOAEL of 0.012 mg γ-HCH/kg/day (Meera et al. 1992), as described in the footnote in Table 2-2.

2.2.2.4 Neurological Effects

In humans, the most commonly reported effects associated with oral exposure to γ-HCH are neurological. Most of the information is from case reports of acute γ-HCH poisoning. No studies were located regarding neurological effects in humans following long-term ingestion of α-, β-, γ-, or δ-HCH. Seizures and convulsions have been observed in individuals who have accidentally or intentionally ingested γ-HCH in insecticide pellets, liquid scabicide, or contaminated food (Davies et al. 1983; Harris et al. 1969; Munk and Nantel 1977; Powell 1980; Starr and Clifford 1972; Storen 1955). In most cases, exposure to γ-HCH was inferred from the presence of γ-HCH in the urine or blood. Also, the actual amount of γ-HCH ingested could not be determined because the γ-HCH was present in solution or in pellets in which other substances were present. Liquid scabicide has been reported to contain approximately 1% γ-HCH (Davies et al. 1983; Powell 1980).

Neurotoxic effects have been reported in several species of animals exposed to γ-HCH. The most serious effects were seizures following a single intragastric administration of approximately 15–60 mg/kg in rats

(Martinez and Martinez-Conde 1995; Martinez et al. 1991; Tilson et al. 1987; Tusell et al. 1987; Vendrell et al. 1992a, 1992b; Woolley and Griffith 1989). Treatment of rats with a single dose of 30 mg lindane/kg by gavage resulted in convulsions 10–30 minutes later, with molecular analysis revealing the repression of calmodulin (CAM) genes, in particular, decreased levels of mRNA from the CAM II gene (Barrón et al. 1995a). Less-serious effects in rats included increased anxiety following a single gavage dose of 20 mg/kg (Llorens et al. 1990b) and increased spontaneous motor behavior observed at 10 mg/kg (Llorens et al. 1989).

Kindling, the induction of seizures with repeated application of subthreshold electrical or chemical stimuli, has been used as a method of investigating neurological response to HCH poisoning. A single oral dose of 5–20 mg lindane/kg to rats previously kindled by electrical stimulus produced incidences of myoclonic jerks and clonic seizures which increased in a dose-dependent manner (Gilbert and Mack 1995). Nonkindled animals displayed these symptoms at a dose of 10 mg lindane/kg. Enhanced susceptibility to kindled seizures brought on by electrical stimulation was seen in rats exposed for 10 weeks to 10 mg lindane/kg/day, 3 days/week (Gilbert 1995). Increased rates of acquisition of kindled seizures were observed following dosing of rats with 3–10 mg lindane/kg/day for 4 days (Joy et al. 1982). An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure to γ-HCH, based on a NOAEL of 1 mg/kg/day for increased kindling acquisition (Joy et al. 1982).

Eleptiform seizures have been reported in male rats fed milk, from dams that were gavaged with 20 mg γ-HCH/kg, on postnatal days 3–15 (Albertson et al. 1985). These data suggest that γ-HCH can be transferred in the dam's milk and elicit neurological effects in offspring. It is not possible to determine the doses received by the pups. Avoidance response latency was statistically increased in rats administered a single dose of 15 mg/kg by gavage (Tilson et al. 1987). No clinical signs of behavioral effects were seen in suckling Wistar rats treated once with 20 mg/kg lindane by gavage at postnatal days 8, 15, 22, or 29, although regional changes in brain noradrenaline and serotonin were seen, with differential effects depending on age at the time of exposure (Rivera et al. 1991).

Changes in levels of brain norepinephrine (Rivera et al. 1991) and serotonin (Attia et al. 1991; Rivera et al. 1991) have also been reported in rats administered acute oral doses of γ-HCH. Decreased dopamine levels were seen in rats treated by gavage with 10 doses totaling 60 mg lindane/kg (half the LC_{50}) over a period of 30 days (Martinez and Martinez-Conde 1995). Increase in the levels of brain catecholamines, particularly norepinephrine and dopamine, and associated signs of toxicity such as mild tremor, lacrimation, salivation, and dysnea were observed in female rats given oral doses of 100 mg/kg/day of technical-grade HCH for

7 days (Raizada et al. 1993). The activity of monoamine oxidase (MAO) in the cerebrum showed a marginal decrease; while the cerebellum and spinal cord indicated a significant increase and decrease in MAO, respectively. Rats treated with 20 mg technical-grade HCH/kg/day in food for 90 days exhibited increased γ-aminobutyric acid (GABA) levels, increased glutamate decarboxylase (GAD) activity, and decreased glutamate levels in the brain (Nagaraja and Desiraju 1994). No significant changes were seen in lipid peroxidation in brain tissue from rats treated for 90 days with 90 mg lindane/kg/day in food, indicating that the tonic convulsions observed throughout the exposure period were probably not brought on by oxidative stress in the brain (Arisi et al. 1994). Decreased myelin basic protein was observed in rats exposed to 5 mg/kg/day by gavage for 3 days (Serrano et al. 1990a).

Longer exposures to lower doses of γ-HCH were reported to result in significantly altered Skinner box behavior (operant conditioning) in a small number of rats exposed to 2.5 mg/kg/day for 40 days (Desi 1974), and significantly decreased nerve conduction velocity in rats exposed to 25.4 mg/kg/day for 30 days (Muller et al. 1981). The latter study did not examine any behavioral parameters.

Similar neurological effects have not been reported in animals treated with α-HCH. Muller et al. (1981) reported no delay in tail nerve conduction velocity in rats fed 5.1, 54.2, or 106.2 mg α-HCH/kg/day for 30 days. However, neurological effects have been reported in rats exposed to β-HCH. Mice treated with 57 or 190 mg/kg/day β-HCH for 30 days developed ataxia within 1 week of treatment (Cornacoff et al 1988). An acute-oral MRL of 0.2 mg/kg/day was derived based on a NOAEL of 19 mg/kg/day for ataxia. The study was limited by small sample size (6 per group) and lack of quantificative and dose-response information. Muller et al. (1981) reported a significant delay in tail nerve conduction velocity in rats fed 66.3 mg β-HCH/kg/day for 30 days. Van Velsen et al. (1986) reported ataxia and coma in rats exposed to 22.5–25 mg β-HCH/kg/day for 13 weeks. Rats treated once with 100 mg δ-HCH/kg by gavage exhibited no convulsions, although molecular analysis revealed a significant decrease in mRNA expression from brain calmodulin (CAM) genes (Barrón et al. 1995). Seizures were noted in mice exposed to technical-grade HCH through feed or gavage at levels of 10–17 mg/kg/day in the feed for 80 weeks (Kashyap et al. 1979). A significant increase in motor activity was noted in rats exposed to technical-grade HCH at a level of 50 mg/kg/day for 120 days (Gopal et al. 1992); a significant decrease in rearing (sitting back on haunches) was seen in rats exposed to 50 mg/kg/day technical-grade HCH and fed a protein-deficient diet. Alterations in neurotransmitter levels, increased brain wave frequency, and behavioral changes were reported in male rats administered 50 mg/kg/day technical-grade HCH by gavage for 1 or 3 months (Anand et al. 1991). Exposure to 0.4 mg/kg/day technical-grade HCH for 360 days resulted in convulsions, tremors, and paralysis in male

rats after 270 days, although the number of animals affected or the severity of the symptoms were not reported (Dikshith et al. 1991a). This study also found degeneration of the cerebellum and cerebellar cortex in animals sacrificed after a one-year exposure to 20 mg/kg/day.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to HCH.

Increased length of estrous cycle and decreased sexual receptivity were found in female rats treated with a single dose of γ-HCH (25 mg/kg) given by gavage (Uphouse and Williams 1989). Inhibition of the formation of estradiol-receptor complex in the rat uterus cytosol was reported in female rats administered 30 mg γ-HCH/kg/day by oral intubation for 7 days (Tezak et al. 1992). Female mink treated with 1 mg/kg/day γ-HCH in their diet from 6 weeks before mating until weaning showed a decrease in receptivity to a second mating and a decrease in whelping rate, although litter size was not affected (Beard et al. 1997). This decreased fertility effect was primarily a result of embryo mortality after implantation. Mouse dams treated with γ-HCH (6.2 mg/kg) during gestation period days 6–12 had increased numbers of resorbed fetuses (Sircar and Lahiri 1989). A lack of implantation sites and pups death were observed following treatment with 10.8 mg/kg/day on gestation days 1–4 and 3.6 mg/kg/day on gestation days 14–19, respectively. Statistically significant increases in the glycogen content of the uterus, cervix, and vagina (but no increase in organ weight) were reported in female rats exposed to 20 mg γ-HCH/kg/day in the diet for 30 days (Raizada et al. 1980). Antiestrogenic properties were found in female rats given oral gavage doses of 10 mg/kg/day γ-HCH for 15 weeks (Chadwick et al. 1988). These responses were not seen at 5 mg/kg/day. Ovariectomized rats exposed for 5 days and sexually immature female rats exposed for 7 days to 40 mg lindane/kg/day showed no effects on the number of estrogen and estrogen-dependent progesterone receptors (Laws et al. 1994). Thus, lindane's antiestrogenic effects in reproductive tissue do not appear to be due to direct action on estrogen receptors or its induction of progesterone receptors. Female rabbits exposed to 0.8 mg γ-HCH/kg/day, 3 days/week for 12 weeks, had a reduced ovulation rate (Lindenau et al. 1994). However, rabbits given the same treatment regime followed by artificial insemination exhibited no effects on the fertilization rate or on pre- or postimplantation losses (Seiler et al. 1994). In male rats, oral administration of 6 mg/kg for 5 days or a single dose of 30 mg/kg of γ-HCH resulted in a reduction in the number of testicular spermatids and epididymal sperms of both treated groups 2 weeks after treatment (Dalsenter et al. 1996). γ-HCH was detected in the testes of both groups 24 hours and 2 weeks after the last treatment. Histological examination by electron microscopy revealed ballooning of the Sertoli cells with

fragmentation or loss of organelles. Similarly, Shivanandappa and Krishnakumari (1983) reported testicular atrophy, degeneration of seminiferous tubules, and disruption of spermatogenesis in male rats fed 75 mg γ-HCH/kg/day for 90 days. Significant reductions in the relative weight of testicles and epididymis, spermatid and sperm counts, and testosterone levels were observed in pubescent or adult rats fed milk as neonates from dams gavaged with 6 mg/kg γ-HCH on lactation day 9 or 14 or 1 mg/kg γ-HCH on lactation days 9–14 (Dalsenter 1997). Histopathological observations included a reduction in Leydig cell numbers and spermatogenesis. However, fertility, measured by impregnation of female rats, was unaffected. Rats exposed to approximately 10 mg/kg/day for 4 generations showed no adverse reproductive effects (Palmer et al. 1978b).

Oral exposure to 60 mg β-HCH/kg for 30 days resulted in normal uteri and reproductive cycling in female mice (Cornacoff et al. 1988). Atrophy of the ovaries and testes, hyperplastic and vacuolized endometrial epithelium, degeneration of the seminiferous tubules, and disruption of spermatogenesis were seen in rats exposed to 22.5–25 mg β-HCH/kg/day in their diet for 13 weeks (Van Velsen et al. 1986). Technical-grade HCH caused transient changes in testes' weights and decreased sperm counts in a 7-week study (Pius et al. 1990), degeneration of seminiferous tubules and Leydig cells (Roy Chowdhury and Gautam 1990), and changes in the muscle layer of the seminiferous tubules (Gautam et al. 1989). None of these studies provide adequate evidence for the effects of technical-grade HCH on sperm function in animals or humans.

In mice, exposure to 90 mg technical-grade HCH/kg/day (isomer composition unknown) for 3 months led to increased testicular weight and degeneration of seminiferous tubules (Nigam et al. 1979). Testicular degeneration was reported in male rats exposed to 20 mg/kg/day technical-grade HCH in the diet for 360 days (Dikshith et al. 1991a). A dose-related increase in fetal resorptions was seen in pregnant female mice treated once with 25–200 mg/kg technical-grade HCH by gavage on the ninth gestation day (Dikshith et al. 1990).

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans following oral exposure to any of the HCH isomers.

A single oral dose of 25 mg/kg technical-grade HCH caused increased resorptions of the fetus in female mice, but fetal development was normal (Dikshith et al. 1990). Srivastava and Raizada (1993) further studied the

prenatal effect of orally administered technical-grade HCH. While mice exposed to HCH during the preimplantation period (day 2–6 of gestation) did not show fetolethality, exposure during the postimplantation period (day 6–12 of gestation) to 25 and 50 mg/kg/day HCH produced significant increases in resorption of fetuses, inhibition of maternal serum progesterone levels, and higher levels of HCH in fetal tissues. Oral exposure to Benesan (a pesticidal formulation containing 50% γ-HCH) given at doses of 6.25, 12.5, or 25 mg/kg/day by gavage on days 6–15 of gestation failed to produce teratogenic effects in rats (Khera et al. 1979). When minks were treated with 1 mg/kg/day γ-HCH in their diet (Beard et al. 1997), the proportion of embryos lost after implantation was increased. In another study, γ-HCH was administered to pregnant mice by gastric intubation on day 12 of gestation. At doses of 30 and 45 mg/kg body weight in C57BL/6J mice, significant decreases in fetal weight, fetal thymic weight, and placental weight were observed (Hassoun and Stohs 1996a). When given to DBA/2J mice at a dose of 45 mg/kg body weight, γ-HCH caused significant reduction in fetal and placental weight. No malformations in the fetuses of both strains of mice were observed, even though the administered doses caused maternal deaths. Increases in the production of lipid metabolites in maternal sera and the amniotic fluids were found to parallel the observed fetotoxicities (Hassoun et al. 1996). Superoxide production, lipid peroxidation and DNA-single strand breaks were increased in fetal and placental tissues 48 hours after administration of single dose of 30 mg/kg γ-HCH to pregnant mice on day 12 of gestation (Hassoun and Stohs 1996b). Significant increases in lipid peroxidation also occurred in fetal livers collected on day 18 of gestation. Thus, it was suggested that fetotoxic effects of γ-HCH may be due to induced oxidative stress, enhanced lipid peroxidation, and DNA-single strand breaks in the fetal and placental tissues of mice. In another study, γ-HCH given to rat dams during gestation and lactation did not cause developmental effects in the pups, but β-HCH (20 mg/kg/day during gestation) caused increased fetal deaths within 5 days of birth and exposure to 5 mg/kg/day during gestation and lactation resulted in increased liver weights of pups (Srinivasan et al. 1991a). When lactating female rats were treated orally with a single dose of 6 mg/kg of γ-HCH on day 9 or 14, or 1 mg/kg on days 9–14 of lactation; the testosterone level of the male offspring was reduced 50% at puberty (day 60) when compared to the control group (Dalsenter et al. 1997a). When the offspring reached adulthood (day 140 postnatal), the relative testicular weight was significantly lower (Dalsenter et al. 1997). The number of sperm and spermatids was also significantly reduced. A dose-related increase in the incidence of fetuses with an extra 14th rib was reported in CFY rats exposed to 5, 10, or 20 mg/kg γ-HCH by gavage during gestation days 6–15; statistical significance was attained only at 20 mg/kg (Palmer et al. 1978a). The incidence of fetuses with an extra 13th rib was statistically increased in rabbits exposed to 20 mg/kg γ-HCH by gavage during gestation days 6–18 (Palmer et al. 1978a). In both rats and rabbits, the incidences of extra ribs were within or just greater than the ranges recorded for the control groups, and therefore, may not be sufficient

evidence of teratogenicity caused by exposure to γ-HCH. No effects on embryonic development were seen in rabbits treated by oral gavage with 0.8 mg lindane/kg, 3 times per week for 12–15 weeks before artificial insemination and throughout gestation (Seiler et al. 1994). Regional changes in brain noradrenaline, serotonin and the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) levels were noted in suckling rats treated with 20 mg/kg/day γ-HCH, as a single dose (Rivera et al. 1991). Alterations in levels of brain dopamine, serotonin, gamma-aminobutyric acid ($GABA_B$), glutamate, glutamate decarboxylase, and noradrenaline were seen in various areas of the brains of female rat pups treated with 10 mg technical-grade HCH/kg/day for 60 days (Nagaraja and Desiraju 1994).

2.2.2.7 Genotoxic Effects

No studies were located regarding genetic effects in humans following oral exposure to HCH.

In animals, ingestion of technical-grade HCH was reported to induce dominant lethal mutations in mice (Lakkad et al. 1982). Oral exposure to α-HCH was reported to result in mitotic disturbances including an increased mitotic rate and an increased frequency of polyploid hepatic cells in rats (Hitachi et al. 1975). Incidence of chromosome clastogeny in bone marrow cells was increased in mice exposed for 7 days to 1.6 mg γ-HCH/kg/day (Kumar et al. 1995).

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding the carcinogenicity of the individual isomers of HCH or technical-grade HCH following ingestion by humans.

α-HCH, β-HCH, γ-HCH, and technical-grade HCH have been shown to be liver carcinogens in rats and mice; however, in some studies the liver was the only organ examined. Ito et al. (1973) examined the carcinogenicity of HCH isomers in dd mice exposed to 45 mg/kg/day of each isomer (total dosage was 90 mg/kg/day) for 24 weeks. Exposure to β-, γ-, or δ-HCH alone did not result in hepatocellular carcinoma. However, when these isomers were mixed with α-HCH, hepatocellular carcinoma was observed. These results suggest that α-HCH is itself a hepatocellular carcinogen or acts synergistically with the other isomers.

In Wistar rats, exposure to 25 mg γ-HCH/kg/day in the diet for 24 or 48 weeks did not result in any identifiable carcinogenic effect (Ito et al. 1975); however, high mortality in the control and treatment groups precludes determination that γ-HCH is not carcinogenic to rats under this experimental protocol. Mice (dd strain) exposed to as much as 90 mg γ-HCH/kg/day in the diet for 24 weeks did not exhibit any carcinogenic effects (Ito et al. 1973). Although an increased incidence of malignant hepatomas was reported in male dd mice exposed to 108–120 mg/kg/day in the diet for 32 weeks (Hanada et al. 1973), this dose level may have exceeded the maximum tolerated dose (MTD), based on effects of γ-HCH on survival. Liver nodules developed in mice receiving 39 mg/kg/day of γ-HCH, although the number of animals tested was small, the study was limited by the lack of statistical analysis.

Information concerning the cancer effects of γ-HCH following chronic-feeding exposure is equivocal. No statistically significant increases in endocrine, thyroid, pituitary, adrenal gland, liver, or ovary tumors were observed in male and female Osborne-Mendel rats fed 10.8–33 mg/kg/day in the diet for 80 weeks (NCI 1977) and in Wistar rats fed 0.07–32 mg γ-HCH/kg/day in the diet for 104 weeks (Amyes 1990); however, poor survival rates limit the significance of these results. On the other hand, hepatocellular carcinomas have been reported in CF_1 and $B6C3F_1$ mice exposed to 13.6–27.2 mg/kg/day in the diet for 80 or 104 weeks, respectively (NCI 1977; Wolff et al. 1987). In addition, hepatocellular carcinomas have been reported in yellow (YS/UY)F-1 mice exposed to 27.2 mg/kg/day in the diet for 96 weeks (Wolff et al. 1987); this strain of mouse has a dominant mutation at the agouti locus (A^{vy}) that results in an increased susceptibility to formation of strain-specific neoplasms. The human oral carcinogenicity assessment for γ-HCH is currently under review (IRIS 1998).

No evidence of liver carcinogenicity was reported in Wistar rats exposed to 45 or 90 mg α-HCH/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975; Nagasaki et al. 1975); high mortality was observed in both the treated and control groups. However, α-HCH appears to be carcinogenic in mice following intermediate-duration exposure. Hepatomas and hepatocellular carcinomas have been reported in a number of strains of mice exposed to 13–95 mg/kg/day for 16–36 weeks (Hanada et al. 1973; Ito et al. 1973, 1976; Nagasaki et al. 1975; Tsukada et al. 1979). Tryphonas and Iverson (1983), however, reported no evidence of a carcinogenic effect in male mice exposed to 90 mg α-HCH/kg/day in the diet for 50 weeks. Ito et al. (1975) reported an increased incidence of hepatocellular carcinoma in male rats exposed to 50 and 75 mg α-HCH/kg/day in the diet for 72 weeks, suggesting that α-HCH may be carcinogenic in rats after long-term exposure. A study of enzyme-altered liver foci in rats treated first with the tumor initiator *N*-nitrosomorpholine, and then 20 mg α-HCH/kg/day in food for 49 weeks, found that the tumor promoter activity of HCH is apparently due to

increased cell proliferation caused by a lowering of the cell death (apoptosis) rate (Luebeck et al. 1995). In another study in rats, additional administration of 35 mg/kg/day of α-HCH in the diet for 65 weeks inhibited the induction of liver tumors by 0.07 mg/kg/day of aflatoxin B_1 (Angsubhakorn et al. 1981). IRIS (1998) lists α-HCH as a probable human carcinogen and estimated an oral cancer potency factor for α-HCH of 6.3 $(mg/kg/day)^{-1}$ based on the incidence of hepatic nodules and hepatocellular carcinomas observed in male mice administered α-HCH in the diet (Ito et al. 1973). The oral cancer potency factor is a plausible upper-bound estimate of the lifetime probability of an individual developing cancer as a result of oral exposure per unit intake of the chemical.

β-HCH has not been found to be carcinogenic in Wistar rats exposed to 25 or 50 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975) or in dd mice exposed to 18–120 mg/kg/day in the diet for 24 or 32 weeks (Hanada et al. 1973; Ito et al. 1973). However, Thorpe and Walker (1973) reported an increased incidence of hepatocellular carcinomas in CF1 mice exposed to 26 mg/kg/day in the diet for 104 weeks. The studies with negative results were, in general, of short duration, used a small number of animals, or failed to examine all of the animals. IRIS (1998) lists β-HCH as a possible human carcinogen and estimated an oral cancer potency factor for ingested β-HCH of 1.8 $(mg/kg/day)^{-1}$ based on the incidence of hepatic nodules and hepatocellular carcinomas observed in male mice administered β-HCH at a single dose level in the diet (Thorpe and Walker 1973). This is the only chronic study from which to estimate cancer risk from exposure to β-HCH. The study is limited by the use of only one nonzero dose group. Also, the use of incidence of liver tumors alone in mice to predict a compound's carcinogenicity in humans may be equivocal (Vesselinovitch and Negri 1988). Diversity of factors has been shown to influence the development of liver cell tumors in mice, such as the strain of the mice (Nagasaki et al. 1975b), the protein or calorific value of the diet (Tannenbaum and Silverstone 1949), and the microbial flora of the animals (Roe and Grant 1970).

δ-HCH has not been found to be carcinogenic in male Wistar rats exposed to 45 or 90 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975) or in male dd mice exposed to 18–90 mg/kg/day in the diet for 24 weeks (Ito et al. 1973). However, these studies were of relatively short-exposure duration. δ-HCH is structurally related to carcinogenic HCH isomers, but it is currently listed as not classifiable for human carcinogenicity (IRIS 1998).

Increased incidence of carcinoma was reported in Swiss mice following exposure to 90 mg technical-grade HCH/kg/day in the diet for 8–32 weeks (Thakore et al. 1981). Increased incidences of hepatocellular carcinoma were also reported in Swiss mice exposed to 21.3–85 mg/kg/day in the diet for 20 months (Munir

et al. 1983) and in Swiss mice exposed to 10 or 17 mg/kg/day through gavage or the diet, respectively, for 80 weeks (Kashyap et al. 1979).

2.2.3 Dermal Exposure

Studies examining the dermal toxicity of HCH in humans are limited. Most of the available information is obtained from cases in which γ-HCH was dermally applied as a scabicide. γ-HCH in topical creams and lotions is efficiently absorbed through the skin (Ginsburg et al. 1977). Although it has been reported that these lotions contain 1% γ-HCH, it is not possible to quantify the amount of γ-HCH to which these individuals were exposed, because of the different areas of skin treated.

2.2.3.1 Death

No studies were located regarding lethal effects in humans following dermal exposure to α-, β-, or δ-HCH. An acute whole-body dermal application of 1% γ-HCH lotion to a 2-month-old infant for the treatment of scabies was reported to result in death (Davies et al. 1983), and a concentration of 110 ppb γ-HCH was identified in the brain. In general, most humans dermally poisoned with γ-HCH have recovered with no apparent adverse effects (Fagan 1981).

In animals, acute dermal exposure to high doses of γ-HCH were reported to result in death. The dermal LD_{50} for γ-HCH is 900 mg/kg in female rats and 1,000 mg/kg in male rats (Gaines 1960). Rats exposed to moistened lindane for 24 hours exhibited no mortality at 250 mg/kg, 20% mortality at 600 mg/kg, 40% mortality at 1,000 mg/kg, and 30% mortality at 2,000 mg/kg (Ullmann 1986a). Significant lethality (47%) was seen in female rats, but not male rats, exposed dermally to 400 mg γ-HCH/kg/day for 13 weeks, 5 days/week, 6 hours/day (Brown 1988). Calves dermally exposed to 33.3 mg/kg γ-HCH died within 5 months (Venant et al. 1991). Dikshith et al. (1978) reported that guinea pigs dermally exposed to 200 mg technical-grade HCH/kg died within 5–12 days. Four of 20 rats died from exposure to technical-grade HCH at 100 mg/kg/day for 15–30 days (Dikshith et al. 1991c). Weanling rabbits were more sensitive to γ-HCH treatment than young adults, as seen by increased mortality rates accompanied by excitement and convulsions after a single whole-body treatment with a 1% solution at a dose of 60 mg γ-HCH/kg (Hanig et al. 1976). This suggests that children might be at a greater risk than adults for toxic responses to dermal absorption of HCH. Rabbits treated with 25 mg/kg/day technical-grade HCH for 30 days by skin painting on shaved dorsal, ventral, or thigh areas exhibited no deaths in the group exposed by dorsal application, but 2 of

8 rabbits died in the group exposed by ventral application, and 4 of 8 died in the group exposed by thigh application (Dikshith et al. 1989b). These and other values are in Tables 2-4 and 2-5.

2.2.3.2 Systemic Effects

Reliable LOAELs for respiratory, hepatic, and renal effects in animals after acute and intermediate exposure to γ-HCH are shown in Table 2-4. Reliable LOAELs for hepatic, renal, and dermal effects in animals after intermediate exposure to technical-grade HCH are shown in Table 2-5.

Respiratory Effects. An acute dermal poisoning of a 2-month-old infant exposed to a whole body application of 1% γ-HCH lotion resulted in death. The autopsy revealed pulmonary petechiae (tiny reddish spots that contain blood) (Davies et al. 1983). Slight dyspnea was observed in rats exposed dermally for 24 hours to 1,000 or 2,000 mg γ-HCH/kg on a shaved patch of dorsal skin (Ullmann 1986a). The dyspnea was severe in one female administered the high dose. Rapid respiration or wheezing was noted in rats exposed dermally to 10 mg γ-HCH/kg/day for 13 weeks (Brown 1988).

Cardiovascular Effects. An acute dermal poisoning of a 2-month-old infant exposed to a whole-body application of 1% γ-HCH lotion resulted in death. The autopsy findings were minimal but revealed epicardial petechiae (Davies et al. 1983).

No studies were located regarding cardiovascular effects in animals following dermal exposure to HCH.

Gastrointestinal Effects. Vomiting and diarrhea occurred in a child who had 1% γ-HCH applied to the skin to treat a rash (Ramchander et al. 1991).

No studies were located regarding gastrointestinal effects in animals following dermal exposure to HCH.

Hematological Effects. Aplastic anemia was documented in a man who applied γ-HCH to his skin for 3 weeks for treatment of scabies (Rauch et al. 1990). Excessive dermal exposure to HCH was reported to result in aplastic anemia and bone marrow hyperplasia in a woman who bathed her dog once a week for 2 years in a preparation that reportedly contained 2% HCH (Woodliff et al. 1966). Reduced hemoglobin and hematocrit values and a nearly complete absence of red blood cell precursors in bone marrow were reported in

TABLE 2-4. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Dermal

Species (strain)	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
ACUTE EXPOSURE						
Death						
Rat (Sherman)	10 d once				1,000 M (LD_{50})	Gaines 1960
					900 F (LD_{50})	
Rat (Wistar)	24 hr once				1,000 (LD_{50})	Ullmann 1986a
Systemic						
Rat (Wistar)	24 hr once	Resp	600	1,000 (dyspnea)		Ullmann 1986a
Rabbit (New Zealand)	once	Ocular		40 (mild eye irratation)		Ullmann 1986c
Rabbit (New Zealand)	4 hr once	Dermal	200			Ullmann 1986d
Neurological						
Rat (Wistar)	24 hr once		600	1,000 (slight sedation)	2,000 F (severe spasms)	Ullmann 1986a
INTERMEDIATE EXPOSURE						
Death						
Rat (Crl:(WI)BR)	13 wk 5 d/wk 6 hr/d		60 F		400 F (23 deaths out of 49)	Brown 1988

TABLE 2-4. Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Dermal (continued)

Species (strain)	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
Systemic						
Rat (Crl:(WI)BR)	13 wk 5 d/wk 6 hr/d	Resp		10 (rapid respiration or wheezing)		Brown 1988
		Hepatic	10	60 (centrilobular hypertrophy)		
		Renal		10 M (hyaline droplet formation		
		Renal	10 F	60 F (basophilic tubules)		
Rat	once for 25 days			180 F (mild dermatitis)		Dikshith et al. 1973
Neurological						
Rat (Crl:(WI)BR)	13 wk 5 d/wk 6 hr/d			10 (hyperactivity)	60 F (ataxia, tremors, convulsions)	Brown 1988

d = day(s); F = female; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s).

TABLE 2-5. Levels of Significant Exposure to Technical - Grade Hexachlorocyclohexane - Dermal

Species (strain)	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL		Reference/ Chemical Form
				Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE						
Death						
Gn pig (NS)	5-12 d 1x/d				200 M (24/24 deaths)	Dikshith et al. 1978 technical
INTERMEDIATE EXPOSURE						
Death						
Rat (Wistar)	15 d 1x/d				100 F (2/10 deaths)	Dikshith et al. 1991c technical
Rabbit (NS)	30 d 1x/d				25 M (6/24 deaths)	Dikshith et al. 1989b technical
Systemic						
Rat (Wistar)	30 d 1x/d	Hepatic			100 F (hypertrophy, fatty degeneration, nuclear pyknosis of hepatocytes, diffuse and focal liver necrosis)	Dikshith et al. 1991c technical
		Renal			100 F (tubular necrosis)	
		Dermal		100F (hyperkeratosis, epidermal cell vacuolization, thickening of collagen fibers)		

TABLE 2-5. Levels of Significant Exposure to Technical - Grade Hexachlorocyclohexane - Dermal (continued)

Species (strain)	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical Form
Rabbit (NS)	30 d 1x/d	Hepatic		25M (hepatocyte degeneration, pycnotic nuclei, enlarged liver, altered GOT, GPT, LDH, and ALP activities)		Dikshith et al. 1989b technical
		Renal			25 M (altered epithelial lining of proximal convoluted tubules, loss of brush borders of tubules, atrophy of glomerular capsules)	
		Dermal		25M (thickened epidermis, hyperkeratinization, and infiltration of mononuclear cells)		
Gn pig (NS)	30 d 1x/d	Hepatic		100M (38% increase in liver weight, hepatic hypertrophy, pycnotic nuclei in cytoplasm, focal fatty inclusions, increased GOT and ALP activity)		Dikshith et al. 1978 technical
		Renal	100 M			
CHRONIC EXPOSURE						
Cancer						
Mouse (Swiss)	80 wk 2 d/wk				2.4 (CEL: liver tumors)	Kashyap et al. 1979 technical

ALP = alkaline phosphatase; CEL = cancer effect level; d = day(s); F = female; Gn pig = guinea pig; GOT = glutamate oxaloacetate transaminase; GPT = glutamate pyruvate transaminase; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s); x = time(s).

a 2-year-old boy exposed to a family dog that was dipped regularly in mange treatment containing 12% γ-HCH (Vodopick 1975).

No studies were located regarding hematological effects in animals following dermal exposure to any of the HCH isomers.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals following dermal exposure to HCH.

Hepatic Effects. No studies were located regarding hepatic effects in humans following dermal exposure to HCH.

Liver pathology, including dilation of sinusoids, focal fatty inclusions, hypertrophy of hepatocytes, thickened blood vessels, swelling, and proliferation of epithelial cells of bile ducts, was observed in guinea pigs treated with 100 mg technical-grade HCH/kg/day for 30 days (Dikshith et al. 1978). The patch of the abdomen on which the HCH was applied was not covered to prevent licking, so oral exposure may also have occurred. In rabbits exposed to 25 mg technical-grade HCH/kg/day for 30 days, there were degenerative changes in hepatocytes along with increased liver and serum GPT and alkaline phosphatase (Dikshith et al. 1989b). Liver cell hypertrophy, fatty degeneration, nuclear pyknosis, and focal and diffuse necrosis were found in female rats treated with 100 mg/kg/day technical-grade HCH for 7–30 days, but the time that it took for these lesions to occur, the severity, and the number of animals affected were not reported (Dikshith et al. 1991c). Centrilobular hypertrophy was reported in male and female rats exposed dermally to 60 mg lindane/kg/day for 13 weeks, 5 days/week, 6 hours/day (Brown 1988).

Renal Effects. No studies were located regarding renal effects in humans following dermal exposure to HCH.

Female rats treated with 100 mg/kg/day of technical-grade HCH for 7, 15, or 30 days had necrosis and atrophy of the renal tubules and glomeruli, although the number of animals affected and the severity of the lesions were not reported (Dikshith et al. 1991c). Similar effects were noted in male rabbits treated with 25 mg/kg/day technical-grade HCH (Dikshith et al. 1989b). Male rats treated dermally with 10 mg/kg/day lindane for 13 weeks exhibited hyaline droplet formation, and urinalysis showed increased cast formation and

positive scores for protein, blood, and turbidity in treated males (Brown 1988). Females in the same study exhibited a slight increase in the incidence of tubular basophilia at 60 mg/kg/day.

Dermal Effects. Rashes were observed in a boy following treatment with shampoo containing γ-HCH (Fagan 1981). No exposure level was reported, but the shampoo was rinsed over the boy's entire body.

Mild dermatitis was observed in rats after 15 skin paintings with 180 mg/kg/day γ-HCH/kg for 25 days (Dikshith et al. 1973). Rabbits exposed to 132 mg/kg moistened lindane for 4 hours showed no primary skin irritation or other toxic symptoms (Ullmann 1986d). Rabbits exposed to technical-grade HCH (25 mg/kg/day for 30 days) had hyperkeratinization of the epidermal layer and swollen collagen fibers in the dermis, but no scoring level was provided (Dikshith et al. 1989b). Dermal treatment of rats with 100 mg/kg/day technical-grade HCH for 7–30 days resulted in hyperkeratosis, epidermal cell vacuolization, and thickening of collagen fibers (Dikshith et al. 1991c).

Ocular Effects. No studies were located regarding ocular effects in humans following dermal exposure to HCH.

Mild eye irritation was seen in rabbits exposed to 26 mg/kg lindane in the conjunctival sac for up to 72 hours, giving a primary irritation score of 0.6 out of a maximum possible cumulative score of 16 (Ullmann 1986c).

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans or animals following dermal exposure to HCH.

2.2.3.4 Neurological Effects

There have been several reports of human intoxication involving convulsions in children after excessive topical application of γ-HCH (Lee and Groth 1977; Matsuoka 1981; Ramchander et al. 1991; Telch and Jarvis 1982; Tenenbein 1991); exposure levels were not reported. Heiberg and Wright (1955) reported convulsions in a woman who had treated calves with an insecticide containing 11% γ-HCH and 16% other HCH isomers. Weakness of the left and right limbs, dysarthria, and dysphagia were seen in an agricultural worker exposed by inhalation and dermal contact to unspecified levels of several organochlorine pesticides,

including lindane (Fonseca et al. 1993). A man with human immunodeficiency virus (HIV) exhibited generalized tonic-clonic seizure activity after a single topical application of a 1% lindane lotion to treat scabies (Solomon et al. 1995).

Studies in animals have substantiated the neurological symptoms resulting from γ-HCH application. Manifestations such as excitability, seizures, and convulsions have been observed in rabbits following a single topical application of 60 mg lindane/kg in a 1% solution (Hanig et al. 1976); young rabbits were more susceptible than older rabbits. Slight sedation was observed in rats exposed once for 24 hours to 1,000 mg/kg lindane through shaved dorsal skin (Ullmann 1986a). Sedation was severe in one female receiving the highest dose (2,000 mg/kg). This female also showed severe spasms. Damage to Purkinje cells in the cerebellum and tremors were found in female Wistar rats treated with 100 mg/kg/day technical-grade HCH for 7–30 days (Dikshith et al. 1991c). Aggressiveness or hyperactivity was noted in female rats exposed dermally for 13 weeks to 10 mg lindane/kg/day, while ataxia and tremors were seen at 60 mg/kg/day (Brown 1988).

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following dermal exposure to HCH. Dikshith et al. (1978) reported testicular hypertrophy and atrophy and complete inhibition of spermatogenesis in guinea pigs dermally treated with technical-grade HCH for 7, 15, or 30 days at doses as low as 100 mg/kg/day. The patch of the abdomen on which the HCH was applied was not covered to prevent licking, so oral exposure more than likely occurred. In a similar study, the backs of male rats were sprayed with 50 or 100 mg/kg/day technical-grade HCH for 120 days and the rats were housed in separate cages to prevent licking (Prasad et al. 1995). Depletion of germ cells and impaired function of Leydig and Sertoli cells was suggested by significant dose-related changes in activities of testicular enzymes such as sorbitol dehydrogenase, glucose-6-P-dehydrogenase, γ-glutamyl transpeptidase, and β-glucoronidase. Significant reductions in sperm count and motility and increased percentages of abnormal sperm were also observed in both groups. A significant reduction in testosterone level was observed in the high dose group.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals following dermal exposure to HCH.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following dermal exposure to HCH.

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

A case-control study surveying childhood brain cancer cases among Missouri residents found that the odds ratios for the use of Kwell, a shampoo containing lindane for lice control, were slightly elevated during the first 7 months of age to diagnosis (Davis et al. 1993). Thus, Kwell use was significantly associated with childhood brain cancer compared to controls. However, this study was limited by small sample sizes, potential recall bias in questionnaires, multiple comparisons, and the lack of detailed exposure information.

In mice, dermal exposure to a 0.5% solution of γ-HCH in acetone applied twice a day for 60 days was reported to result in no treatment-related tumors (Orr 1948). Increases that were not statistically significant were reported in the incidences of hyperplastic and preneoplastic areas in the liver and hepatic tumors in Swiss mice exposed to 2.4 mg technical-grade HCH/kg/day for 80 weeks (Kashyap et al. 1979). Limitations of these studies, including less-than-lifetime exposure and study duration, the testing of only one dose, and the potential for ingestion of some of the compound from the skin, preclude determination that dermally applied HCH is noncarcinogenic in mice.

2.3 TOXICOKINETICS

Absorption of the various HCH isomers following inhalation, oral, or dermal exposure has been inferred from humans who have become ill or who had increased serum levels of the various isomers following exposure by these routes. No animal data are available from the inhalation route to quantify the extent or rate of absorption. Technical-grade HCH has been shown to be well absorbed from the gastrointestinal tract of animals (Albro and Thomas 1974). The distribution of HCH isomers in humans and animals is primarily to the adipose tissue but also to the brain, kidney, muscle, blood, and other tissues (Siddiqui et al. 1981a; Baumann et al. 1980). β-HCH accumulates to a much greater extent than γ-HCH. The excretion of HCH isomer metabolites is primarily through the urine. The isomers have also been detected in breast milk (Ejobi et al.

1996; Schoula et al. 1996) and semen (Szymczynski et al. 1981) . The primary urinary metabolites are chlorophenols and an epoxide. The conversion occurs mainly by the action of hepatic enzymes.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Evidence exists that humans absorb γ-HCH vapor or dusts via inhalation. This can be inferred from occupational studies in which adverse health effects, including hematological abnormalities and neurological effects, have been reported in workers exposed to γ-HCH in workplace air (Brassow et al. 1981; Czegledi-Janko and Avar 1970; Kashyap 1986; Samuels and Milby 1971). In addition, α-, β-, γ-, and δ-HCH have been detected in the blood serum, adipose tissue, and semen of occupationally and environmentally exposed individuals indicating that absorption does take place (Baumann et al. 1980; Czegledi-Janko and Avar 1970; Kashyap 1986; Nigam et al. 1986; Saxena et al. 1980, 1981a, 1981b). There are no specific studies that have quantified the rate or extent of absorption of the HCH isomers following inhalation exposure. No information is available on the absorption of α-, β-, γ-, and δ-HCH following inhalation exposure in experimental animals.

2.3.1.2 Oral Exposure

In humans, HCH is absorbed following oral exposure. Many accidental poisonings have occurred in humans as a result of γ-HCH ingestion, and high blood concentrations have been demonstrated in a number of acute poisoning cases (Berry et al. 1987; Harris et al. 1969; Khare et al. 1977; Munk and Nantel 1977; Nantel et al. 1977; Powell 1980; Starr and Clifford 1972).

HCH is similarly absorbed following oral exposure in animals. Information concerning the rate of absorption from the gastrointestinal tract can be inferred from studies conducted in mice and rats. These studies indicated that γ-HCH is readily absorbed from the gastrointestinal tract (Ahdaya et al. 1981; Turner and Shanks 1980). Ahdaya et al. (1981) demonstrated that half of the administered dose was absorbed from the gastrointestinal tract of fasting mice approximately 14 minutes after administration of radiolabelled γ-HCH by stomach tube. Although this study demonstrates the rapid absorption of γ-HCH from the gastrointestinal tract, the use of fasted animals prevents an assessment of the effect of stomach contents on the rate of absorption. Turner and Shanks (1980) studied the rate of absorption of γ-HCH from the gastrointestinal

tract and intestinal lymphatic system using rat intestinal loop preparations. Prepared loops were injected with γ-HCH, and the blood and lymph were sampled for 30 minutes. γ-HCH was readily absorbed from the intestine into the blood; however, only a small amount of γ-HCH entered the lymphatic system from the intestine.

Absorption of technical-grade HCH following oral exposure has been quantified in rats. The extent of absorption of technical-grade HCH has been estimated to be 95.8% in rats within 4 days following the oral administration of single doses of the substance (Albro and Thomas 1974). Variation of the dosages from 30 to 125 mg/kg had no effect on the percentage of absorption. The overall degree of absorption of technical-grade HCH administered in the feed for 14 days was similar (94.9%), but the average absorption values of α-, β-, γ-, and δ-HCH were 97.4%, 90.7%, 99.4%, and 91.9%, respectively (Albro and Thomas 1974).

2.3.1.3 Dermal Exposure

The ready absorption of γ-HCH across human skin, due to its lipid solubility, has been demonstrated in several studies that examined the absorption of γ-HCH from an antiscabies lotion (Feldmann and Maibach 1974; Lange et al. 1981; Franz et al. 1996). Maximum serum levels in healthy volunteers and scabies patients were reported within 4–6 hours following whole-body application (Lange et al. 1981). However, the maximum serum levels of γ-HCH in scabies patients were greater than those reported for normal volunteers. Studies involving a single topical application of γ-HCH to the forearm, which was left for 24 hours before washing, indicate that at least 9% of the applied dose was absorbed; maximum absorption occurred during the first 12 hours after application of γ-HCH to the skin, but absorption continued for at least 5 days (Feldmann and Maibach 1974).

The absorption of γ-HCH through the skin was studied following application of 2 different preparations to the forearm of human volunteers (Dick et al. 1997a). One with 120 mg γ-HCH/ml in acetone as the vehicle and the other, a commercial product, consisted of 3 mg γ-HCH/ml formulation which primarily contained white spirit as the solvent base. The proportion of the applied dose absorbed into the systemic circulation in 6 hours was 5% for the dose applied in acetone and 60% of the applied dose in white spirit-based formulation. Thus, the white spirit enhanced the absorption of γ-HCH relative to acetone as the vehicle. The absorption of γ-HCH through human skin was also assessed in an *in vitro* study (Dick et al. 1997b). γ-HCH absorption was reported to be 15–25% in 24 hours for the 2 formulations that contained white spirit as the

predominant solvent, 3% in 24 hours from an aqueous spray dilution, and <1% in 24 hours for the acetone preparation.

γ-HCH is similarly absorbed through the skin of animals. Toxicity was observed in guinea pigs and rabbits following dermal exposure to γ-HCH and following dermal exposure to technical-grade HCH (Dikshith et al. 1978; Hanig et al. 1976). Male rats treated dermally with radiolabelled lindane (20% emulsifiable concentrate) on a 4.9 cm^2 shaved dorsal area exhibited absorption of radiolabel which increased with time of exposure (Bosch 1987a). After 4 hours, 10.1%, 5.3%, and 2.0% were absorbed from doses of 0.06, 0.6, and 6 $mg/cm^2/kg$, respectively. After 24 hours, 27.7%, 20.9%, and 5.1% were absorbed from doses of 0.06, 0.6, and 6 $mg/cm^2/kg$, respectively. Male rabbits treated dermally with radiolabelled lindane (20% emulsifiable concentrate) in a 28.3-cm^2 shaved dorsal area absorbed, after 4 hours, 29.6%, 18.3%, and 7.3% radiolabel from doses of 0.005, 0.05, and 0.5 $mg/cm^2/kg$, respectively, and, after 24 hours, 55.7%, 40.0%, and 16.6% from the same respective doses (Bosch 1987b).

The absorption of γ-HCH in infants and children who had received dermal treatment with 1% lindane(γ-HCH) lotion was investigated in one study (Ginsburg et al. 1977). Maximum blood concentrations were observed in 6 hours, and averaged at 0.028 μg/ml for the group infected with scabies and 0.024 μg/ml for the noninfected group.

2.3.2 Distribution

Placental transfer of HCH in humans has been well documented (Saxena et al. 1981a). The levels of HCH and other organochlorine insecticides were found to be higher in the maternal blood, placenta, and umbilical-cord blood of stillborn cases than those of live-born cases (Saxena and Siddiqui 1983). HCH has been shown to accumulate in amniotic fluid, placenta and fetal tissues after oral treatment of pregnant mice (Srivastava and Raizada 1993) and can be related to fetolethality. HCH isomers have been detected in human breast-milk, particularly in developing countries that still use HCH as a pesticide. Detected concentrations in these studies are discussed in Section 5.6. In a study on rats, γ-HCH has been reported to be transferred in the breastmilk and to elicit neurological effects in neonates. Epileptiform seizures have been reported in male rats fed maternal milk for 12 days beginning on the third day after birth, from dams exposed daily to 20 mg γ-HCH/kg by gavage (Albertson et al. 1985). In another study, lactating females were treated orally with a single dose of 6 mg/kg of γ-HCH on day 9 to 14 of lactation, the testosterone level of the male offspring was reduced 50% when puberty was reached (day 60) when compared to the control group (Dalsenter et al. 1997).

When the offspring reached adulthood (day 140 postnatal), the relative testicular weight was significantly lower (Dalsenter et al. 1997). The number of sperm and spermatids was also significantly reduced. α-, β-, and γ-HCH have been found to be bioconcentrated and excreted in women's breast milk who have been exposed to technical-grade HCH in pesticide residues (Nair et al. 1996).

2.3.2.1 Inhalation Exposure

Information on the distribution of the HCH isomers, following inhalation by humans, comes from studies of humans exposed to HCH in the workplace. Air concentrations of α-HCH (0.002–1.99 mg/m^3), β-HCH (0.001–0.38 mg/m^3), and γ-HCH (0.004–0.15 mg/m^3) were associated with concurrent mean blood serum levels in workers of 69.6, 190.3, and 36.9 µg/L, respectively (Baumann et al. 1980). Serum levels of total HCH of 0.14–0.60 ppm were found in workers with unknown levels of exposure to technical-grade HCH (Nigam et al. 1986). HCH isomers have also been detected in the adipose tissues of workers occupationally exposed and individuals exposed via the ambient environment (Baumann et al. 1980; Siddiqui et al. 1981a). Accumulation of β-HCH has been shown to increase approximately linearly with time of exposure (Baumann et al. 1980). Siddiqui et al. (1981a) found adipose levels of 0.1–1.5, 0.06–0.9, 0.7–3.0, and 0.97–5.8 ppm of α-, β-, γ-, and total HCH, respectively, in the tissues collected during an autopsy case study conducted in India.

In a study with Wistar rats exposed to air concentrations of 0.02–5 mg/m^3 lindane for 90 days, male rats exhibited higher serum lindane levels than females, but females had higher liver, brain, and fat levels (Oldiges et al. 1983). The organ levels of lindane were dose-dependent but had returned to baseline levels after a 4-week recovery period.

2.3.2.2 Oral Exposure

Information on the distribution of the HCH isomers following ingestion by humans comes from case reports. A fatal poisoning case confirmed that γ-HCH is, in part, distributed to the central nervous system. γ-HCH was detected in the cerebrospinal fluid of a young boy following ingestion of an unknown quantity of γ-HCH (Davies et al. 1983).

More detailed information on the distribution of HCH or its isomers is available from studies in which laboratory animals were exposed by ingestion (Chand and Ramachandran 1980; Eichler et al. 1983;

Srinivasan and Radhakrishnamurty 1983b). These studies examined the overall distribution pattern of HCH isomers. γ-HCH and β-HCH are primarily stored in the fat of rats acutely exposed for 5, 10, or 15 days (Srinivasan and Radhakrishnamurty 1983b). The overall distribution of γ-HCH was greatest in fat, followed by brain, kidney, muscle, lungs, heart, spleen, liver, and blood. More recently, γ-HCH has also been found in the adrenal glands of rats (Lahiri et al. 1990; Sulik et al. 1988). In an experiment lasting 12 days, the accumulation of γ-HCH in the brain of rats gavaged with 5 or 12 mg/kg/day began to decline after 8 days. This reduction was not observed in rats gavaged with 20 mg/kg/day (Tusell et al. 1988). In rats gavaged with γ-HCH on lactation day 9 or 14, γ-HCH levels were higher in their milk than plasma (Dalsenter et al. 1997). Levels of γ-HCH in the offspring of those rats were approximately twice as high in kidneys and liver than in brain and testes. In the brain of rats, α-HCH has been found to accumulate preferentially in the white matter, an area containing lipid-rich myelin, as opposed to gray matter (Portig et al. 1989). However, the same brain distribution pattern was not noted for γ-HCH in mice, despite the fact that it is equally lipophilic. Differences in distribution of γ-HCH and α-HCH are most likely due to stereospecific forces.

The distribution pattern for β-HCH was found to be in the following order: fat > kidney > lungs > liver > muscle > heart > spleen > brain > blood. For γ-HCH, the distribution pattern was as follows: fat > brain > kidney > muscle > lungs > heart > spleen > liver > blood. β-HCH accumulates in tissues to a greater degree than γ-HCH except in the brain, where the γ-HCH accumulates at a higher concentration (Srinivasan and Radhakrishnamurty 1983b). This accumulation increases with increasing dose and treatment period for β-HCH more so than for γ-HCH. The greater accumulation of β-HCH in tissues is expected since this isomer is known to be metabolized more slowly. In addition, γ-HCH is known to induce the liver mixed-function oxygenase system, and thus. self-induced metabolism is an important factor that minimizes the accumulation of γ-HCH residues in animal tissues.

The preferential accumulation of HCH in fatty tissues is also observed following intermediate-duration exposure of rats to HCH (isomer unspecified) in the diet (overall distribution: fat > liver > serum) (Chand and Ramachandran 1980) or exposure to α- or γ-HCH by gavage (overall distribution: fat > kidney > liver > brain > blood) (Eichler et al. 1983).

2.3.2.3 Dermal Exposure

Information on the distribution of the HCH isomers in exposed humans comes from case reports. A fatal poisoning case indicated that γ-HCH is, in part, distributed to the brain following topical application. The isomer was detected in brain tissue (110 ppb) and heart blood (33.3 ppb) collected during the autopsy of an infant who was treated with a whole-body application of a 1% γ-HCH lotion after a hot bath (Davies et al. 1983). In another study, blood levels of γ-HCH peaked 6 hours following topical application of a 1% solution to 20 children (12 infected with scabies, 8 noninfected) (Ginsburg et al. 1977). Mean concentrations did not differ statistically between the two groups at 6 hours and were 0.024 µg/ml in healthy children and 0.028 µg/ml in infected children. The half-life in blood was 17.9 hours and 21.4 hours in infected and healthy children respectively. Differences in dosage between the two groups of children were considered marginally significant (p=0.11). However, the infected children were younger. The mean age for the infected and noninfected group were 32.5 months and 64.3 months, respectively.

The distribution of γ-HCH through the skin was studied following application of 2 different preparations to the forearm of human volunteers (Dick et al. 1997a). The mean peak plasma concentrations of γ-HCH following exposure to the acetone and white-spirit based applications were 0.91 and 0.47 ng/mL, respectively; although the preparation in acetone contained a 40-fold higher concentration of γ-HCH. About 30% of the applied dose for the white-spirit based formulation was observed in the stratum corneum at 6 hours exposure and decreased by 90% at 24 hours. Fifteen percent of the applied dose for the acetone-based application was located in the stratum corneum.

Some information on the distribution of γ-HCH is available from studies in which laboratory animals were exposed by dermal application (Bosch 1987a, 1987b; Hanig et al. 1976; Solomon et al. 1977a, 1977b). A study on the distribution of γ-HCH in guinea pigs following acute dermal exposure indicates that accumulation of γ-HCH in the brain is greater than in the blood after single and multiple topical applications (Solomon et al. 1977a, 1977b); the levels in both tissues increased with the number of applications. Experiments with radiolabeled lindane in dermally treated rats (Bosch 1987a) and rabbits (Bosch 1987b) found that absorption of radiolabel increased with time of exposure, with greater absorption and subsequent excretion in the urine occurring at the lower treatment doses. In weanling rabbits, which appear to be more sensitive to lindane toxicity from dermal exposure than young adults, levels of lindane in the blood after a single application of a 1% solution (60 mg lindane/kg) were 1.67 and 2.48 µg/mL in 2 individuals that had been shaved and depilated, then stripped to remove the keratin layer (Hanig et al. 1976). In contrast, a blood level of only 0.67 µg/mL was seen in an individual that had only been shaved and depilated, indicating that absorption increases with loss of skin integrity.

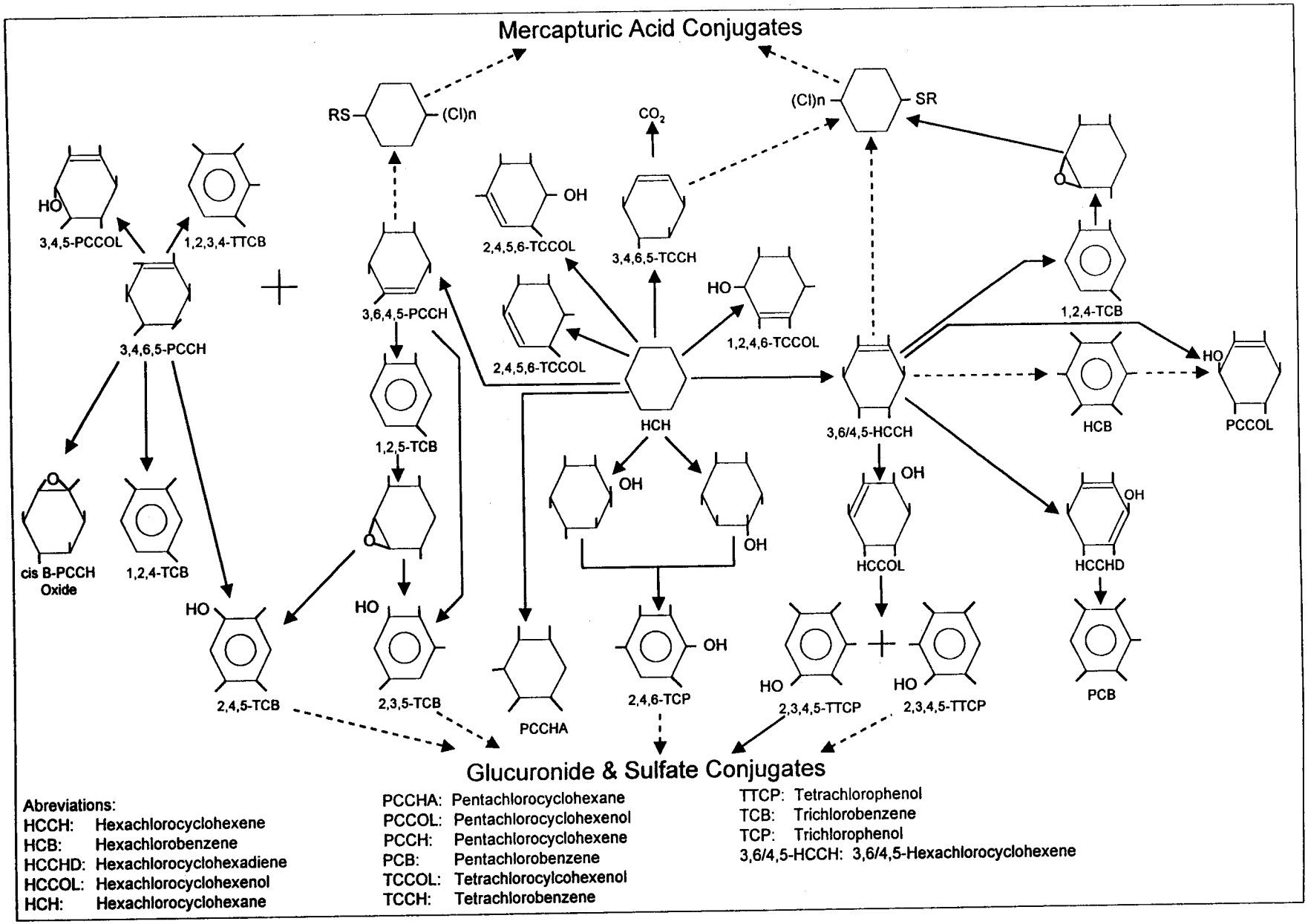
Following dermal treatment of rats with 50 or 100 mg/kg/day technical-grade HCH for 120 days, α-, β-, γ-, and δ-HCH were accumulated in testicular tissue and sperm in a dose-related manner (Prasad et al. 1995). β-HCH was present at the highest concentration in testicular tissue and sperm.

2.3.3 Metabolism

The metabolism of γ-HCH is illustrated in Figure 2-4. Angerer et al. (1983) determined that chlorophenols were the primary urinary metabolites of γ-HCH excreted by workers involved in γ-HCH production. In the study, glucuronides and sulfates of chlorophenols were cleaved by acidic hydrolysis of urine samples. The metabolites 2,3,5-, 2,4,6-, and 2,4,5-trichlorophenol accounted for almost 57.7% of the γ-HCH metabolites identified in the urine collected during the last 2 hours of the workers' shifts. Other urinary metabolites identified included other trichlorophenols, dichlorophenols, tetrachlorophenols, and dihydroxychlorobenzenes. Pentachlorophenol has also been identified as a urinary metabolite in humans following occupational exposure (Engst et al. 1979). *In vitro* investigations indicate that human liver microsomes convert γ-HCH by dechlorination, dehydrogenation, dehydrochlorination, and hydroxylation to 5 primary metabolites: 3,6/4,5-hexachlorocyclohexene, pentachlorocyclohexene, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorobenzene (Fitzloff et al. 1982). Similar *in vitro* studies have demonstrated that an epoxide forms during the metabolism of pentachlorocyclohexene. This stable halogenated hydrocarbon epoxide metabolite may be responsible for the mutagenic and carcinogenic effects of γ-HCH (Fitzloff and Pan 1984).

In animals, γ-HCH appears to be transformed by hepatic enzymes to form chlorophenols, chlorobenzene, chlorocyclohexanes, chlorocyclohexanols, and conjugates of mercapturic acid, glucuronide, and sulfate (Chadwick and Freal 1972a; Chadwick et al. 1978a; Engst et al. 1979; Kujawa et al. 1977). These metabolites have been identified in various tissues and in the urine of laboratory animals. Metabolites found in the liver of rats following intermediate exposure to γ-HCH via gavage or diet include di-, tri-, tetra-, and pentachlorobenzenes; pentachlorocyclohexenes; and pentachloro-2-cyclohexen-1-ol (Chadwick and Freal 1972a; Kujawa et al. 1977). Metabolites identified in the blood of these rats include di-, tri-, tetra-, and pentachlorophenols and pentachloro-2-cyclohexen-1-ol (Kujawa et al. 1977). Di-, tri-, and tetrachlorophenols; pentachlorocyclohexenes; and pentachloro-2-cyclohexen-1-ol have been identified in samples of kidney, spleen, heart, and brain tissue from rats fed γ-HCH (Kujawa et al. 1977). Metabolites found in the urine include tri-, tetra-, and pentachlorophenol; pentachloro-2-cyclohexen-1-ol; and isomers of tetrachloro-2-cyclohexen-1-ol (Chadwick and Freal 1972a; Chadwick et al. 1978c; Kujawa et al. 1977). The metabolism of γ-HCH in the intestine was reported to be very minor, or the metabolites were completely

Figure 2-4. The Proposed Metabolism of Hexachlorocyclohexane*



*Adapted from Chadwick et al. 1979, 1985; Fitzlof and Pan 1984; Fitzloff et al. 1982

absorbed. No metabolites were detected in the feces or in the adrenal gland (Kujawa et al. 1977). *In vitro* preparations using rat liver slices have also found that γ-HCH is converted to hexachlorobenzene (Gopalaswamy and Aiyar 1984). However, these findings have not yet been confirmed in *in vivo* experiments.

The major urinary metabolites formed in rats, following intermediate oral exposure to α- or β-HCH, were identified as tri- and tetrachlorophenols; pentachlorocyclohexene was also identified as a metabolite of γ-HCH in kidney tissue (Macholz et al. 1982a, 1982b).

The detoxification of γ-HCH appears to be dependent on the P-450 oxidative system. Intermediate exposure to lindane resulted in greater toxicity in DBA/2 (D2) mice than in C57BL/6 (B6) mice; the former are unresponsive to microsomal enzyme induction by lindane (Liu and Morgan 1986). Increased toxicity was associated with higher blood and brain concentrations in D2 mice than in B6 mice at the time of sacrifice. In addition, D2 mice were found to have more 2,4,6-trichlorophenol in the liver, kidney, and spleen than the less-susceptible B6 mice. The inability of D2 mice to undergo enzyme induction to increase the rate of detoxification led to γ-HCH's enhanced toxicity in this strain. Other investigators have demonstrated the importance of the hepatic microsomal enzymes in the detoxification of γ-HCH (Baker et al. 1985; Chadwick and Freal 1972a; Chand and Ramachandran 1980; Chadwick et al. 1981; Tanaka et al. 1979). Chadwick et al. (1981) demonstrated that pretreatment of rats with inducers of hepatic enzymes significantly influenced the metabolism and excretion of γ-HCH and its metabolites by altering specific metabolic pathways; excretion of γ-HCH metabolites in the urine increased nearly 4-fold following pretreatment with Aroclor 1254 or phenobarbitol. Following pretreatment with Aroclor 1254, a 7-fold increase in expired metabolites was observed. Naphthoflavon had no effect on excretion rate.

Metabolism of HCH has not been studied in children. However, although it is unknown whether the ability to metabolize HCH specifically differs between children and adults, some enzymes which belong to the enzyme superfamilies involved in phase II HCH metabolism are developmentally regulated in humans. The development of UDP-glucuronosyltransferase (responsible for glucuronide conjugation) depends on the enzyme isoform but in general adult activity is attained by 6–18 months of age (Leeder and Kearns 1997). Development of sulfotransferase (responsible for sulfate conjugates) activity is also substrate specific and is usually earlier than UDP-glucuronosyltransferase. In fact, levels of some sulfotransferases may be greater during infancy and early childhood than during adulthood (Leeder and Kearns 1997). A series of enzymes are involved in the production of mercapturic acid conjugates: γ-glutamyltranspeptidase, glutathione

S-transferase, cysteinyl glycinase, and N-acetyl transferase (Sipes and Gandolfi 1991). There are 2 superfamilies of N-acetyltransferases, and one—the N–acetyltransferase 2 superfamily—has members that are developmentally regulated in humans. There is some N-acetyltransferase 2 activity in fetuses by 16 weeks of gestation. Infants up to 2 months of age have the slow metabolizer phenotype (there is a genetic polymorphism in this enzyme in adults). The adult distribution of slow and fast metabolizer phenotypes is reached by 4–6 months of age and full adult activity is achieved at 1–3 years of age (Leeder and Kearns 1997).

2.3.4 Elimination and Excretion

Excretion of hexachlorocyclohexane has not been studied in children.

2.3.4.1 Inhalation Exposure

Humans excrete γ-HCH and its metabolites in urine, milk, and semen (Angerer et al. 1981). Chromatographic analysis of urine from humans occupationally exposed to HCH showed the presence of chlorinated phenols and all isomers of di-, tri-, and tetrachlorophenol (Angerer et al. 1981). In another study, the elimination of β-HCH was investigated in a group of 40 former workers of a γ-HCH-producing plant by analyzing at least 2 blood specimens from different time points between 1952 and 1980. The median half-life of β-HCH was 7.2 years, calculated by concentrations in whole blood, and 7.6 years, calculated by concentrations in extractable lipids (Jung et al. 1997), assuming first order kinetics for excretion. HCH is commonly detected in low concentrations (0.015 mg/kg fat) in the breastmilk of women exposed to HCH in the environment (Fytianos et al. 1985). All five of the HCH isomers discussed in this profile have been detected in human semen following environmental exposure, suggesting another route of elimination (Szymczynski and Waliszewski 1981). No animal studies using the inhalation route of exposure were located.

2.3.4.2 Oral Exposure

Excretion of γ-HCH and its metabolites in laboratory animals has been well documented. Data indicate that its major route of elimination is via the urine following intermediate and chronic oral feeding in mice (Chadwick et al. 1985). Very little is eliminated in exhaled air (Ahdaya et al. 1981; Chadwick et al. 1985) or

in feces (Chadwick et al. 1985) following acute, intermediate, and chronic oral administration in rodents. Because of its high lipid solubility, γ-HCH is excreted through the dam's milk (Dalsenter et al. 1997).

Very little γ-HCH is excreted unaltered. Various phenylmercapturic acid derivatives have been detected in the urine of rats, formed by the conjugation of γ-HCH metabolites with glutathione subsequent to dechlorinations and dehydrochlorinations (Allsup and Walsh 1982; Kurihara et al. 1979). *In vitro* investigations using rat liver cells indicate that β-HCH seems to resist, to some extent, conversion to the glutathione derivative; γ-HCH and α-HCH are readily conjugated (Fitzloff and Pan 1984; Fitzloff et al. 1982). γ-HCH derivatives are not only excreted in the form of phenylmercapturic acids; there is ample evidence that they are also excreted in the form of glucuronides and sulfate conjugates (Chadwick et al. 1978a).

2.3.4.3 Dermal Exposure

Nonmetabolized γ-HCH was excreted in the urine and feces of healthy volunteers and scabies patients acutely exposed to a 0.3% γ-HCH emulsion by whole-body application. The cumulative excretion of nonmetabolized γ-HCH was almost the same in the healthy volunteers and the scabies patients (Zesch et al. 1982).

The elimination of γ-HCH was studied following application of two different preparations to the forearm of human volunteers (Dick et al. 1997a). The elimination half-life was between 50–111 hours for the acetone-based application, and 25–58 hours for the white-spirit based formulation. Absorbed γ-HCH was excreted in the urine as conjugates of 2,4,6-; 2,3,5-; and 2,4,5-trichlorophenol. Only 0.01–0.15% of the dose was excreted in the urine in 72 hours following dermal exposure for 6 hours.

In a study in which children infected with scabies and their noninfected siblings were treated dermally with 1% lindane lotion, the blood level was found to diminish rapidly after application, with a half-life of 17.9 hours in infected children and 21.4 hours in noninfected children.

In male rats treated dermally with radiolabeled lindane, 0.28, 0.08, and 0.02% radiolabel were excreted in urine 4 hours after doses of 0.06, 0.6, and 6 mg/cm^2/kg, respectively (Bosch 1987a). After 24 hours, 4.4, 3.2, and 0.6% radiolabel were excreted in urine from the same respective doses. In a similar study with male rabbits, 3.8, 2.6, and 1.3% radiolabel were excreted in urine 4 hours after doses of 0.005, 0.05, and

0.5 mg/cm^2/kg, respectively (Bosch 1987b). After 24 hours, 25.5, 11.6, and 6.8% radiolabel were excreted in urine from the same respective doses.

2.3.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically-sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-5 shows a conceptualized representation of a PBPK model.

If PBPK models for hexachlorocyclohexane exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

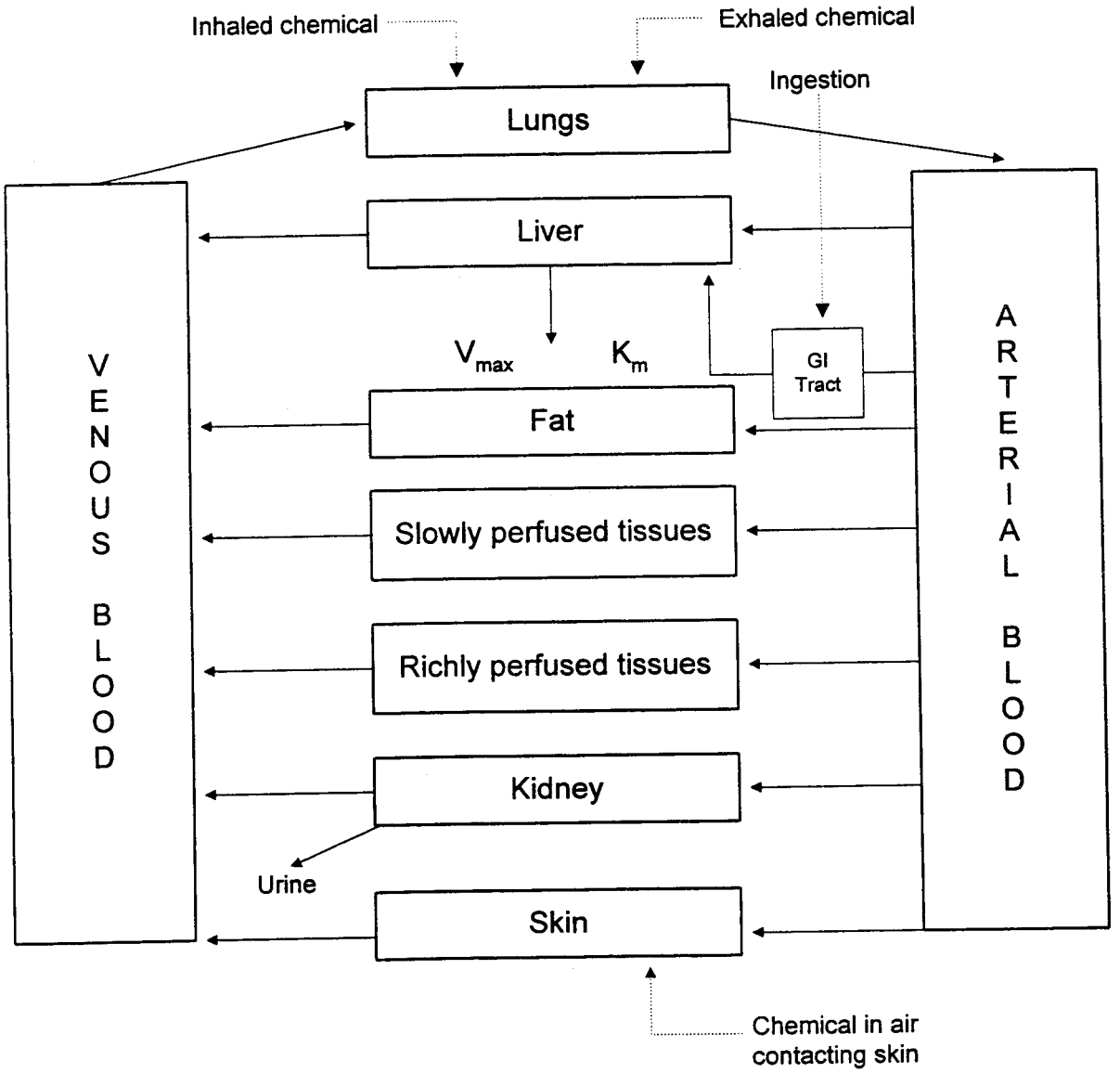
An existing PBPK model for hexachlorocyclohexane is discussed below.

2.3.5.1 Summary of PBPK Models.

DeJongh and Blaauboer (1997) simulated the kinetics of lindane in rats with a PBPK model. A five compartment model for the rat as presented in Figure 2-6 was constructed, including (1) the liver, serving as the metabolizing organ; (2) blood; (3) fat; (4) brain; and (5) a lumped compartment representing all other tissues, consisting mainly of muscle tissue. Values for the physiological parameters, tissue-blood partition coefficients, were obtained from the literature and are presented in Table 2-6. The model was calibrated on a dataset from the literature on the disposition of lindane from blood *in vivo* after single oral dosage and first order biotransformation and gastrointestinal absorption constants for lindane were obtained.

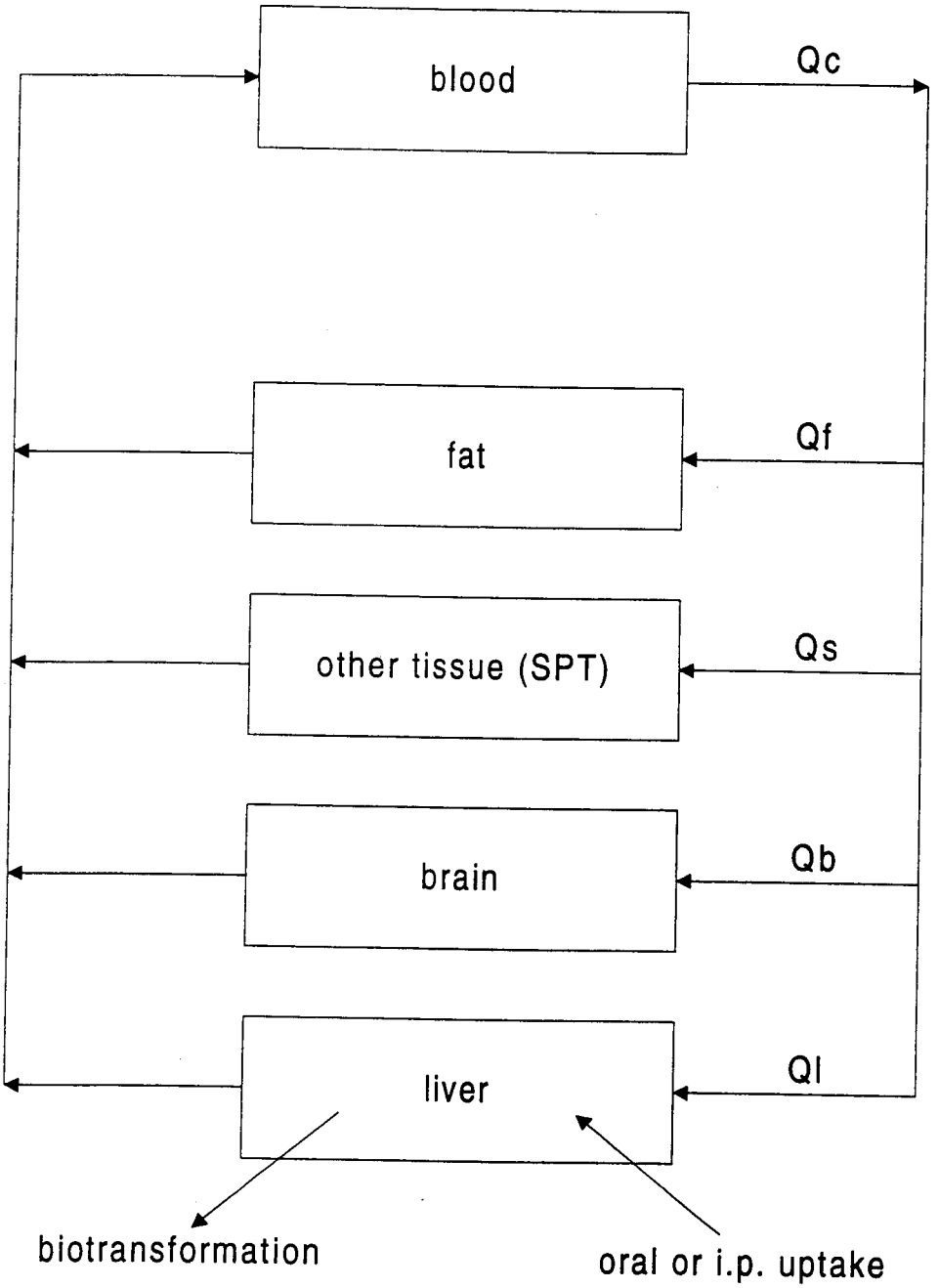
The model was validated by simulating the disposition of lindane *in vivo* after single intraperitoneal and chronic oral dosage and comparing simulated with experimental results. Simulated lindane concentrations in blood, brain, muscle, and fat after single intraperitoneal and chronic oral dosage compared adequately well with experimental results.

Figure 2-5. Conceptual Representation of a Physiologically-Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically -based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Figure 2-6. PBPK Model for Gamma-Hexachlorocyclohexane



Qc = movement from blood to other tissues
Qf = uptake to fat
Qs = uptake to other tissues
Qb = uptake to brain
Ql = uptake to liver

Table 2-6. Parameters for a PBPK Model for γ-Hexachlorocyclohexane in Rats

Parameter		Value	Scaling factor
Body weight (kg)		0.135-0.313	
—Cardiac output	(L/h kg)[a]	14	
$BW^{0.74}$			
Blood flow fractions[a]			
Liver		0.25	—
Fat		0.09	—
Other tissues (SPT)		0.63	—
Brain		0.03	—
Tissue group volume fractions			
Blood[a]		0.06	—
Liver[a]		0.04	—
Brain[a]		0.0006	—
Fat[b]		0.2 x BW + 0.0166	—
Remaining tissues (SPT)		0.894-VFC	—
Partition coefficients for toluene			
Liver-blood[c]		4.2	—
Fat-blood[c]		95.3	—
SPT-blood[c]		1.6	—
Brain-blood[d]		4.1	—
Metabolic and uptake constants			
Biotransformation rate[e]	(h^{-1} kg^{-1})	4.5	$BW^{-0.3}$
Oral/intraperitoneal uptake rate[e]	(h^{-1})	0.035	—
Oral/intraperitoneal uptake efficiency[d]		0.8	—

VFC, relative adipose tissue mass where VFC=0.2*BW + 0.0166
SPT, slowly perfused tissue.
[a] Reference values (Arms and Travis, 1988).
[b] Calculated as a function of body weight (Bailey et al., 1980).
[c] Measured *in vitro* (Jepson et al., 1994).
[d] Measured *in vivo* (Oshiba, 1972).
[e] Value obtained by calibration

There are no PBPK models for HCH in children.

Currently, ATSDR is assessing the feasibility of using tools such as physiologically based pharmacokinetic modeling and pharmacodynamic modeling to extrapolate data across routes or durations of exposure. ATSDR acknowledges that such extrapolation may be done on a substance-by-substance basis after adequate toxicokinetic information has been collected.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Information is available to assess the extent and rate of HCH absorption following oral and dermal exposure (Ahdaya et al. 1981; Albro and Thomas 1974; Turner and Shanks 1980). However, inhalation absorption of HCH can only be inferred from toxicity studies and studies assessing the distribution and excretion of γ-HCH. No quantitative information is available to assess the rate and extent of inhalation absorption. Additional data concerning the absorption of HCH in animals may provide information to assist in characterizing absorption of HCH in humans.

2.4.2 Mechanisms of Toxicity

In the nervous system, γ-HCH is thought to interfere with γ-aminobutyric acid (GABA) neurotransmitter function by interacting with the $GABA_A$ receptor-chloride channel complex at the picrotoxin binding site (Abalis et al. 1985; Anand et al. 1998; Casida and Lawrence 1985; Lawrence and Casida 1984; Pomès et al. 1994; Anand et al. 1998). Thus, the seizures caused by γ-HCH can be antagonized by $GABA_A$ mimetics. The δ-HCH isomer has also been shown to act at the picrotoxin binding site, but to a lesser extent (Fishman and Gianutsos 1988). In rat cortical neurons, expression of the protooncogene *c-fos*, which is associated with seizure activity and is induced by elevated intracellular calcium levels, was increased by γ-HCH treatment but decreased by δ-HCH treatment (Barrón et al. 1995). Treatment-related changes in *c-fos* expression suggested that γ-HCH induces seizures through the activation of calcium channels, while inhibition of calcium channels by δ-HCH results in anticonvulsant effects. The α-HCH isomer, another nonconvulsant, has been shown, like δ-HCH, to suppress c-*fos* induction (Vendrell et al. 1992a). In a study on the cytotoxic action of δ-HCH and γ-HCH in cultured rat cerebellar granule neurons (Rosa et al. 1997), both isomers were found to induce an increase in the free intracellular Ca^{2+} concentration. However, the γ-isomer mainly caused

this increase by a release from intracellular Ca^{2+} stores. On the other hand, δ-HCH may exert its action by stimulating a large influx of Ca^{2+}. δ-HCH was found to be more potent and active as a cytotoxic agent than γ-HCH, and the differences in cytotoxicity and neurotoxic action may be related to their action on the different Ca^{2+} pools. Other suggestive data concerning mechanisms by which HCH causes neurological effects in animals include enhanced synaptic activity (Joy 1982; Joy and Albertson 1985), altered GABA functional activity (Bhatt and Panchal 1994; Cattabeni et al. 1983; Fishman and Gianutsos 1987, 1988; Hulth et al. 1978; Joy and Albertson 1985), and inhibition of Na^{+}-K^{+}-ATPase (McNamara and Krop 1948a; Nakajima 1983; Uchida et al. 1974).

Lindane interacts with cellular membranes and may produce several generalized cytotoxic effects associated with impaired membrane function. In rat renal cortical tubules, glucose uptake and cyclic AMP accumulation were altered by lindane treatment (López-Aparicio et al. 1994). Transport of D-galactose and L-leucine across enterocytes was decreased in chickens injected daily with lindane for 7 days (Moreno et al. 1994). Rats exposed orally to 3.6 mg/kg/day technical-grade HCH for 3–6 months exhibited significantly decreased levels of phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate in the erythrocyte membrane and cerebrum (Agrawal et al. 1995).

Oxidative stress in the liver has been suggested as a mechanism of γ-HCH-induced hepatotoxicity (Azzalis et al. 1995; Barros et al. 1988, 1991; Jungueira et al. 1993; Puri and Kohli 1995; Srinivasan and Radhakrishnamurty 1983a; Videla et al. 1991). This condition is characterized in the rat liver by a reduction in hepatic glutathione content, lipid peroxidation, the microsomal generation of superoxide radical coupled to cytochrome P-450 induction, and a decrement in superoxide dismutase and catalase activity (Jungueira et al. 1997). However, species differences exist in the activities of hepatic metabolizing enzymes, and it has been demonstrated that γ-HCH at a dose of 10 mg/kg/day for 6 days increased the hepatic cytochrome P-450 as well as glutathione-*S*-transferase in the rat, but not in the rabbit or monkey (Puri and Kohli 1995). Thus, oxidative stress and hepatotoxicity are produced with γ-HCH treatment in rats, but not in the rabbit and monkey (Puri and Kohli 1995). Inhibition of Mg^{2+}-ATPase activity has also been observed in rat liver tissue, suggesting an ATPase enzyme sensitivity to the action of γ-HCH (Gopalaswamy and Aiyar 1984). The researchers suggested that some toxic effects appearing in mammals as a result of γ-HCH exposure may arise from its influence on this ATPase activity (Gopalaswamy and Aiyar 1984).

Delayed vaginal opening and disrupted estrous cycle in female Fischer 344 rats and reduced embryo implantation in mice following γ-HCH treatment have been discussed as evidence of antiestrogenic activity

by γ-HCH(Chadwick et al. 1988; Cooper et al. 1989; Sircar and Lahiri 1989). This is in contrast to a previous study indicating estrogenic activity of γ-HCH based on increased glycogen content of the uterus, cervix, and vagina (Raizada et al. 1980). Also, in another study, β-HCH mobilized from fat during fasting produced estrogenic effects and stimulated growth of the uteri in ovariectomized mice (Bigsby et al. 1997). Inconsistencies in the classification of estrogenic activity for γ-HCH may have been due to variations in experimental protocols, examination of different endpoints, and controversy in the interpretation of hormonal effects (Chadwick et al. 1988).

2.4.3 Animal-to-Human Extrapolations

Extrapolating animal toxicity data to predict human risk from HCH exposure appears to be reasonable since similar effects are seen in both test subjects. However, caution must be exercised in animal-to-human extrapolation because of differences in metabolism, toxicokinetics, and mechanisms of toxicity.

Exceptions in extrapolation may include kidney damage in the male rat by γ-HCH (Dietrich and Swenberg 1990, 1991) via α-2μ-globulin, a protein that is not present in humans.

2.5 RELEVANCE TO PUBLIC HEALTH

Issues relevant to children are explicitly discussed in 2.6 Children's Susceptibility and 5.6 Exposures of Children.

Overview

Evidence was found in the reviewed literature that HCH isomers are toxic to humans and animals. Human exposure to HCH occurs primarily by occupational exposure, by ingesting HCH in contaminated food or water, or through the use or misuse of therapeutic lotions containing γ-HCH to control mites or lice. Humans are generally exposed to γ-HCH or to technical-grade HCH, which contains α-, β-, γ-, and δ-HCH. Technical-grade HCH and α-, β-, and δ-HCH isomers are currently unavailable in the United States; therefore, exposure to these isomers is likely to occur only in or near sites at which technical-grade HCH was disposed. Humans can absorb HCH following inhalation, ingestion, or dermal exposure. The possible human health effects associated with exposure to HCH are adverse hematological effects, hepatic effects, renal effects, immunological effects, neurological effects, reproductive effects, and cancer. These effects are

strongly dependent on dose, duration of exposure, route of administration, and the isomer to which the individual is exposed.

Minimal Risk Levels for α-, β-, γ-, and δ-HCH

Inhalation MRLs

No MRLs could be developed because of the lack of data.

Oral MRLs

- An MRL of 0.2 mg/kg/day has been derived for acute-duration (14 days or less) oral exposure to β-HCH. This was based on a NOAEL of 19 mg β-HCH/kg/day for neurotoxic effects (i.e., ataxia) in mice (Cornacoff et al. 1988). In this study, female B6C3F_1 mice were treated with β-HCH in the diet for 30 days. Mice receiving 57 or 190 mg/kg/day exhibited ataxia within 1 week. No ataxia was seen at 19 mg/kg/day.

- An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure to γ-HCH. This was based on a NOAEL of 1 mg γ-HCH/kg/day for neurological (i.e., no increased kindling acquisition) effects in male rats (Joy et al. 1982). In this study, kindling (the development of seizure with repeated application of initially subthreshold electrical stimuli) was examined. Increased kindling acquisition was noted at 3 or 10 mg/kg/day dose levels but not at 1 mg/kg/day.

- An MRL of 0.0006 mg/kg/day has been derived for intermediate-duration oral exposure to β-HCH. This was based on a LOAEL of 0.18 mg β-HCH/kg/day for liver effects in rats (Van Velsen et al. 1986). In this study, hyalinization of centrilobular cells was observed at the low dose of 0.18 mg/kg/day. At 4.5 mg/kg/day (a higher dose), hepatic effects consisted of centrilobular hepatocytic hypertrophy, focal liver cell necrosis, and proliferation of smooth endoplasmic reticulum.

- An MRL of 0.00001 mg/kg/day has been derived for intermediate-duration oral exposure to γ-HCH. This was based on a LOAEL of 0.012 mg γ-HCH/kg/day for immunological/lymphoreticular effects in female mice (Meera et al. 1992). Effects consisted of a dose-dependent biphasic response, i.e.,

stimulation followed by suppression, in cell-mediated and humoral components of the immunological profile. A NOAEL for this effect was not established.

- An MRL of 0.008 mg/kg/day has been derived for chronic-duration (365 days and longer) oral exposure to α-HCH. This was based on a NOAEL of 0.8 mg/kg/day for liver effects in male and female rats (Fitzhugh et al. 1950). Hepatic effects at the 4 mg/kg/day dose level in this study included a significant increase in liver weight and slight microscopic liver damage, i.e., diffuse cell enlargement, focal necrosis, and fatty degeneration.

No acute-, intermediate-, or chronic-duration oral MRL's were derived for technical-grade HCH. HCH is not found in the environment as technical-grade HCH, and analytical methods do not detect or measure technical-grade HCH, but rather, the individual isomers. When technical-grade HCH is accidentally spilled into the environment, individual isomers partition into various media at different rates depending on the physical characteristics of each isomer. Some isomers may be more mobile in soil or water than others. Differences in partitioning and degradation would result in a different proportion of isomers than when initially spilled. Therefore, the development of an MRL for technical grade HCH would not be of value.

Death. Exposure to excessive amounts of HCH, primarily γ-HCH, by inhalation or ingestion, has been reported to result in death in humans (Loge 1965; Mobbs 1948; Storen 1955; Sunder Ram Rao et al. 1988). Doses of γ-HCH and β-HCH which have caused death in animals have been reported for acute oral and dermal exposures and intermediate-duration oral exposures. The acute lethality of HCH in animals may be related to its effects on the central nervous system since convulsions and coma were often observed prior to death. The doses associated with death and increased mortality in animals are much higher than would be caused by HCH in water or soil surrounding waste sites, so it is not likely that humans would die following brief or prolonged exposure to HCH in food, water, or soil.

Systemic Effects

Respiratory Effects. Mucous membrane irritation has been reported in humans exposed to a γ-HCH vaporizer (Conley 1952). Respiratory effects have not been reported in animal studies involving HCH inhalation (Klonne and Kintigh 1988; Oldiges et al. 1983). Whole-body, fatal, dermal exposure of an infant to 1% γ-HCH produced pulmonary petechiae (Davies et al. 1983). Rapid respiration or wheezing was seen in rats exposed dermally to 10 mg lindane/kg/day for 13 weeks (Brown 1988).

Cardiovascular Effects. Electrocardiogram abnormalities were seen in some workers exposed by inhalation to HCH (Kahyap 1986). Whole-body, fatal, dermal exposure of an infant to 1% γ-HCH produced epicardial petechiae (Davies et al. 1983).

Gastrointestinal Effects. Humans who ingested γ-HCH-contaminated food experienced vomiting, nausea, loss of appetite, and diarrhea (Nantel et al. 1977). Vomiting and diarrhea were also seen in a child dermally exposed to a 1% γ-HCH solution (Ramchander et al. 1991). Oral treatment with γ-HCH has inhibited enzyme activity in rat jejunum (Moreno et al. 1996).

Hematological Effects. Blood disorders, including anemia, leukopenia, leukocytosis, granulocytopenia, granulocytosis, eosinophilia, monocytosis, pancytopenia, and thrombocytopenia have been observed in individuals exposed to γ-HCH at work or in homes where HCH vaporizers were operated (Brassow et al. 1981; Danopoulos et al. 1953; Friberg and Martensson 1953; Gewin 1939; Jedlicka et al. 1958; Loge 1965; Mendeloff and Smith 1955; Morgan et al. 1980; Rugman and Cosstick 1990; Samuels and Milby 1971). Ingestion of γ-HCH has resulted in disseminated intravascular coagulation (Sunder Ram Rao et al. 1988). Dermal exposure to γ-HCH has resulted in anemia, bone marrow hyperplasia, and reduction of blood cell precursors in bone marrow (Rauch et al. 1990; Vodopick 1975; Woodliff et al. 1966). No significant hematological effects were reported in 40 workers exposed to γ-HCH in an occupational setting (Milby and Samuels 1971). There are no studies that examine hematological effects in animals following dermal exposure. Oral exposure to γ-HCH had no effect on hematological parameters in dogs and rats (Rivett et al. 1978; Suter 1983), but oral exposure in mice resulted in a reduction of bone marrow precursor cells (Hong and Boorman 1993). Oral exposure of rats to β-HCH resulted in reduced numbers of erythrocytes and leukocytes and decreased hemoglobin concentration and packed cell volumes (Van Velsen et al. 1986). Hematological effects were not observed in rats following inhalation exposure to γ-HCH (Oldiges et al. 1983). Ingestion of technical-grade HCH resulted in decreased white blood cell counts in rats (Joseph et al. 1992c).

Human data suggests that γ-HCH has the potential to induce adverse hematological effects but establishing a causal relationship has been difficult due to a lack of personal exposure data. Animals appear to be less sensitive to γ-HCH but comparison between humans and animals is difficult because limited data is available.

Musculoskeletal Effects. Seizures, limb muscle weakness, and necrosis were seen in humans who ingested γ-HCH powder (Munk and Nantel 1977; Sunder Ram Rao et al. 1988). Decreased cross-sectional bone area was seen in young rats treated with 20 mg/kg/day γ-HCH by gavage for 10 weeks (Andrews and Gray 1990). Suppression of marrow progenitor cells was seen in mice exposed to 10 mg/kg/day lindane for 10 days (Hong and Boorman 1993). In humans, severe poisoning with lindane can result in rhabdomyolysis (necrosis of skeletal muscle) with subsequent myoglobin release, resulting in secondary renal failure (Munk and Nantel 1977; Sunder Ram Rao et al. 1988).

Hepatic Effects. Hepatic effects, such as increased liver enzymes, have been reported in individuals exposed to technical-grade HCH principally by inhalation in a pesticide formulating plant (Kashyap 1986); there is no data reported for individuals who ingested HCH or applied γ-HCH to their skin. An increase in cytochrome P-450 concentration has been reported in rats following inhalation exposure (Oldiges et al. 1983). In animal experiments, ingestion of α-, β-, and γ-HCH and technical-grade HCH was reported to result in some degree of liver toxicity including increased microsomal activity, increased liver weight, mild-to-moderate liver necrosis and fatty degeneration, and liver cancer (Fitzhugh et al. 1950; Hanada et al. 1973; Ito et al. 1973, 1975, 1976; Kashyap et al. 1979; Munir et al. 1983; Nagasaki et al. 1975; NCI 1977; Oesch et al. 1982; Ortega et al. 1957; Thakore et al. 1981; Thorpe and Walker 1973; Tryphonas and Iverson 1983; Tsukada et al. 1979). Often, biochemical or gross changes were not accompanied by histopathological changes. Hepatic effects in animals, following dermal exposure to lindane and technical–grade HCH, were similar to those observed with oral exposure (Dikshith et al. 1978, 1989b, 1991c; Brown 1988). Available human studies are limited, but effects on liver enzymes following exposure to technical-grade HCH were similar to those observed in animal studies. The observation of serious hepatic effects in animals suggests that the same results could potentially occur in workers following prolonged occupational exposure.

Renal Effects. Evidence of renal dysfunction has not been observed in humans exposed to HCH by any route. However, renal effects including increased kidney weight, glucosuria, calcification, and nephritis have been reported in animals exposed to technical-grade HCH and α-, β-, γ-HCH in the diet (Fitzhugh et al. 1950; Srinivasan et al. 1984; Van Velsen et al. 1986). Studies from one laboratory (Dietrich and Swenberg 1990, 1991; Dikshith 1989a, 1991a; Philip et al. 1989a) indicate that the mechanism for renal toxicity of γ-HCH in Fischer-344 male rats may be based on rat α-2μ-globulin, a protein that does not occur in humans or female rats. However, female rats treated dermally with technical-grade HCH had demonstrated renal toxicity (Dikshith et al. 1991c). Thus, renal effects of HCH cannot be all attributed to α-2μ-globulin

interaction. However, at higher doses, lindane can produce lysis of the skeletal muscle with subsequent myoglobinuria, resulting in secondary kidney failure (Munk and Nantel 1977; Sunder Ram Rao et al. 1988).

Dermal Effects. The use of shampoo containing γ-HCH has resulted in skin rashes in humans (Fagan 1981). Dermatitis was seen in rats after daily skin paintings with 180 mg γ-HCH/kg/day for 15–25 days (Dikshith et al. 1973). Rabbits exposed to technical-grade HCH (25 mg/kg/day for 30 days) had hyperkeratinization of the epidermal layer and swollen collagen fibers in the dermis (Dikshith et al. 1989b). However, rabbits exposed to 132 mg/kg moistened lindane for 4 hours showed no primary skin irritation or other toxic symptoms (Ullmann 1986d).

Ocular Effects. Mice exposed to lindane aerosol (5 mg/m^3) for 14 weeks exhibited no ophthalmic effects (Klonne and Kintigh 1988). Mild eye irritation was seen in rabbits exposed to 26 mg/kg lindane in the conjunctival sac for up to 72 hours (Ullmann 1986c). No ophthalmoscopic effects were seen in rats exposed dermally to lindane (up to 400 mg/kg/day) for 13 weeks (Brown 1988).

Body Weight Effects. No effects on body weight in humans exposed to HCH by any route have been seen. No body weight effects were seen in rats exposed to up to 5 mg/m^3 lindane aerosol for 90 days (Oldiges et al. 1983). Significantly decreased body weight gain has been seen in rats treated orally with α- (Fitzhugh et al. 1950), β- (Fitzhugh et al. 1950; Van Velsen et al. 1986), γ- (Fitzhugh et al. 1950; Laws et al. 1994), and technical-grade HCH (Gautam et al. 1989; Joseph et al. 1992b; Nagaraja and Desiraju 1994).

Metabolic Effects. No metabolic effects in humans exposed to HCH by any route have been seen. Increased phosphoinositide turnover and generation of second messengers from phosphoinositides were seen in erythrocyte membranes from female rats treated by gavage with a single dose of 100 mg/kg technical-grade HCH, or with doses of 5 mg/kg/day technical-grade HCH for 3–6 months, 5 days/week (Agrawal et al. 1995). The latter exposure regime also resulted in a significant decrease in phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate in erythrocyte membrane and cerebrum, and levels decreased with increased time of treatment (3–6 months).

Other Systemic Effects. No other systemic effects have been observed in humans or animals exposed to HCH by any route.

Immunological and Lymphoreticular Effects. A significant increase in the level of IgM was observed in workers exposed to technical-grade HCH (Kashyap 1986). Although there is no evidence of an increase in immunoglobulins in animals, antibody response has been reported to be depressed in rats, rabbits, and mice exposed to γ-HCH (Banerjee et al. 1996; Desi et al. 1978; Dewan et al. 1980). Biphasic effects on immunosuppression were reported in mice fed γ-HCH (Meera et al. 1992). This is suggestive evidence that HCH may affect the human immune system.

Neurological Effects. In humans, neurological effects including paresthesia of the face and extremities, headaches, vertigo, abnormal EEG patterns, and often seizures and convulsions have been reported in individuals occupationally exposed to γ-HCH or in individuals exposed accidentally or intentionally to large amounts of γ-HCH by ingestion or dermal application (Czegledi-Janko and Avar 1970; Davies et al. 1983; Harris et al. 1969; Heiberg and Wright 1955; Kashyap 1986; Lee and Groth 1977; Matsuoka 1981; Munk and Nantel 1977; Nantel et al. 1977; Powell 1980; Ramchander et al. 1991; Solomon et al. 1995; Starr and Clifford 1972; Telch and Jarvis 1982; Tenebein 1991). Acute- and intermediate-duration exposure of animals to high oral or dermal doses of γ- or β-HCH affects the central nervous system as evidenced by behavior disorders, decreased nerve velocity, convulsions, seizures, and coma (Albertson et al. 1985; Desi 1974; Dikshith et al. 1991c; Hanig et al. 1976; Muller et al. 1981; Tilson et al. 1987; Tusell et al. 1987; Van Velsen et al. 1986; Vendrell et al. 1992a, 1992b; Woolley and Griffith 1989). No histological examinations were conducted on the brain or nervous system of animals exposed by any route for any duration. The effects in humans and in animals suggest that exposure of humans to high air concentrations or large oral doses could result in neurotoxic effects.

Reproductive Effects. Alterations in reproductive hormones and increased blood levels of γ-HCH and total-HCH isomers have been detected in women that have undergone spontaneous abortion and premature delivery and have been reported in men occupationally exposed to γ-HCH and total-HCH isomers as well as to other organochlorine pesticides (Saxena et al. 1981a; Tomczak et al. 1981; Wasserman et al. 1982). The results of the Saxena et al. (1980, 1981a) studies suggest that pregnant women exposed to organochlorine pesticides, including γ-HCH, were at a greater risk for premature labor and/or abortion. The biological significance of altered hormonal levels in humans is difficult to assess, although the data do suggest that HCH may potentially affect reproductive capability. Similar reproductive hormonal effects have not been reported in animals. However, histological effects on the testes and uterus have been observed in rats orally exposed to high doses of β-HCH, γ-HCH, or technical-grade HCH (Dalsenter et al. 1996; Nigam et al. 1979;

Prasad et al. 1995; Raizada et al. 1980; Van Velsen et al. 1986) and in male rats fed milk from γ-HCH-treated dams (Dalsenter et al. 1997).

Although exposure by injection of γ-HCH in humans is unlikely, reproductive effects have been observed in animals exposed to γ-HCH by this route. Female rats exhibited decreased sexual receptivity after exposure to a 33 mg/kg intraperitoneal injection (Uphouse 1987). In addition, testicular changes including decreased organ weight and degeneration of tubules were observed after 10 daily exposures to 4 mg/kg intraperitoneal injections (Roy Chowdhury et al. 1987). Finally, 10 mg/kg intratesticular injections for 10 days resulted in hypertrophic or atrophic changes (Dikshith and Datta 1972). A three-generation feeding study in rats revealed no adverse reproductive effects from daily doses of approximately 10 mg/kg (Palmer et al. 1978a).

Developmental Effects. Although there are no data regarding developmental effects in humans via any route of exposure, there are animal data on developmental effects. A dose of 30 mg/kg γ-HCH administered to mice on day 12 of gestation caused decreases in fetal weight, fetal thymic weight, and placental weight (Hassoun and Stohs 1996a). The only consistent finding is for extra ribs, which is considered a normal variation and not a toxic effect (Palmer 1978a). However, dams that were exposed to 20 mg/kg/day β-HCH had pups that died within 5 days of birth (Srinivasan et al. 1991a). γ- and technical-grade HCH have altered neurotransmitter levels in rat offsprings (Rivera et al. 1991; Nagaraja and Desiraju 1994).

Genotoxic Effects. The available genotoxicity data indicate that γ-HCH and its individual isomers have some genotoxic potential, but the evidence for this is not conclusive.

γ-HCH has been tested in several *in vitro* genotoxicity studies. In bacteria, it was not observed to induce gene mutations in assays with or without a metabolic activation system (Moriya et al. 1983; Nagy et al. 1975), and it did not produce DNA damage, although a mammalian metabolic activation system was not present (Shirasu et al. 1976). γ-HCH was also not mutagenic in yeast (Shahin and von Borstel 1977) or algae (Kar and Singh 1979a). Mitotic disturbances (c-mitosis which is characterized by spindle breakdown as that produced by colchicine) and chromosome aberrations were observed in onion root tip cells exposed to commercial γ-HCH (Nybom and Knutsson 1947). In mammalian cells, γ-HCH (purity not reported) induced a marginal increase in the frequency of chromosome aberrations (including chromosomal gaps) in Chinese hamster cells, which was interpreted by the authors of the study as providing suggestive, but not conclusive, evidence of an effect (Ishidate and Odashima 1977). γ-HCH (NTP 1984) and technical-grade lindane (Murli 1990) were both reported to be negative for cytogenetic effects in Chinese hamster ovary cells. Technical-

grade lindane was also found inactive for inducing unscheduled DNA synthesis in rat primary hepatocytes *in vitro* (Cifone 1990). α-HCH and γ-HCH were reported to bind to calf thymus DNA in the presence of metabolic activation (Iverson et al. 1984). Cultured human lymphocytes taken from three healthy males showed a dose-dependent increase in chromosomal aberrations (gaps, breaks, and fragments) with significant increases at 0.1 µL/mL technical-grade HCH (6.5% γ-HCH) for 48-hour treatment and at 0.05 and 0.1 µL/mL for 72-hour treatment (Rupa et al. 1989d). In addition, sister chromatid exchanges increased in a dose-dependent manner with the high dose (0.1 µL/mL) producing the only significant result. These results suggest mild mutagenic activity at high doses in humans (Rupa et al. 1989d).

γ-HCH has also been tested *in vivo* in animals. Technical-grade HCH was reported to induce dominant lethal mutations in mice (Lakkad et al. 1982). It did not induce chromosome aberrations in bone marrow cells of Syrian hamsters (Dzwonkowska and Hubner 1986), but positive results were reported in bone marrow cells of rats exposed to β-HCH (Shimazu et al. 1972). γ-HCH was negative in a micronucleus assay in mice (Jenssen and Ramel 1980). α-HCH increased the mitotic rate and frequency of polyploid cells in rat hepatocytes (Hitachi et al. 1975). α-HCH produces DNA fragmentation in primary cultures of rat and human hepatocytes, but not in mouse hepatocytes (Mattioli et al. 1996). DNA repair induction was absent in hepatocytes from all three species. Both α- and γ-HCH have been observed to bind to mouse liver DNA at a low rate (Iverson et al. 1984).

Tables 2-7 and 2-8 present the results of *in vivo* and *in vitro* genotoxicity studies on γ-HCH.

Cancer. Use of γ-HCH pesticides by farmers was associated with a 50% increased risk of non-Hodgkin's lymphoma (Blair et al. 1998). However, a causal relationship could not be determined due to confounding effects such as use of other pesticides. With oral exposure, α-HCH, β-HCH, γ-HCH, and technical-grade HCH have been found to be carcinogenic in mice following long-term exposure (Hanada et al. 1973; Ito et al. 1973, 1975, 1976; Kashyap et al. 1979; Munir et al. 1983; Nagasaki et al. 1975; NCI 1977; Thakore et al. 1981; Thorpe and Walker 1973; Tsukada et al. 1979; Wolff et al. 1987). Hepatocellular carcinoma is the most frequently reported tumor type, although in many studies the liver was the only organ under investigation. In general, mice appear to be more susceptible to the carcinogenic effects of HCH isomers; rats generally developed cancer following longer exposure or exposure to higher doses. In addition, Schroter et al. (1987) reported that α-, β-, and γ-HCH promoted tumor development in rats exposed to a single dose of *N*-nitroso-morpholine. The available animal data suggest that liver cancer may be of potential concern to individuals exposed to HCH isomers for prolonged periods of time.

TABLE 2-7. Genotoxicity of Hexachlorocyclohexane Isomers *In Vivo*

Species (test system)	End point	Results	Isomer	Reference
Mammalian cells:				
Human (peripheral lymphocytes)	Chromosomal aberrations	-	Gamma	Kiraly et al. 1979
Syrian hamster (bone marrow)	Chromosomal aberrations	-	Gamma	Dzwonkowska and Hubner 1986
Rat (bone marrow)	Chromosomal aberrations	+	Beta	Shimazu et al. 1972
Mouse (germ cells)	Dominant Lethal	+	Technical	Lakkad et al. 1982
Mouse	Micronuclei	-	Gamma	Jenssen and Ramal 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Gamma	Kumar et al. 1995
Mouse (liver)	DNA binding	(+)	Alpha/Gamma	Iverson et al. 1984
Rat (liver)	Mitotic disturbances	+	Alpha	Hitachi et al. 1975

+ = positive result; - = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid

TABLE 2-8. Genotoxicity of Hexachlorocyclohexane Isomers *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Isomer	Reference
Prokaryotic organisms:					
Salmonella typhimurium (TA100, TA98, TA1535, TA1537, TA1538)	Gene mutation	-	-	Gamma	Moriya et al. 1983
Escherichia coli (WP2/spot test)	Gene mutation	NT	-	Gamma	Nagy et al. 1975
E. coli (WP2 *hcr*)	Gene mutation	-	-	Gamma	Moriya et al. 1983
Bacillus subtilis (rec⁻ assay)	DNA damage	NT	-	Gamma	Shirasu et al. 1976
Eukaryotic organisms:					
Fungi and plant cells:					
Saccharomyces cerevisiae	Gene mutation	-	-	Gamma	Shahin and von Borstel 1977
Nostoc muscorum	Gene mutation	NT	-	Gamma	Kar and Singh 1979a
Allium cepa	Mitotic disturbances	NT	+	Gamma	Nybom and Knutsson 1947
Mammalian cells:					
Human (SV-40 fibroblasts)	Unscheduled DNA synthesis	-	-	Gamma	Ahmed et al. 1977
Human (peripheral lymphocytes)	Unscheduled DNA synthesis	NT	+	Gamma	Rocchi et al. 1980
Human (peripheral lymphocytes)	Sister chromatid exchange	NT	+	Technical	Rupa et al. 1989d
Human (peripheral lymphocytes	Chromosomal aberrations	NT	+	Technical	Rupa et al. 1989d
Chinese hamster (CHL cells)	Chromosomal aberrations	NT	(+)	Gamma	Ishidate and Odashima 1977
Calf (thymus DNA)	DNA binding	(+)	NT	Alpha/Gamma	Iverson et al. 1984

+ = positive result; - = negative result; NT = not tested; (+) = weakly positive result; DNA = deoxyribonucleic acid

A metabolite of γ-HCH, 2,4,6-trichlorophenol, accounts for 10–20% of γ-HCH-derived excretion products and may be responsible for some or all of the carcinogenic activity observed in mice. 2,4,6-Trichlorophenol has been classified by EPA as a group B2 compound based on evidence of its carcinogenicity in animal test systems (IRIS 1993). Similarly, a stable halogenated epoxide of the γ-HCH metabolite, pentachlorocyclohexene, may be responsible for the carcinogenic effects of γ-HCH (Fitzloff and Pan 1984). Pentachlorocyclohexene has been identified in the liver of rats exposed to γ-HCH (Chadwick and Freal 1972a; Engst et al. 1976; Kujawa et al. 1977). *In vitro* investigations indicate that human liver microsomal enzymes can convert γ-HCH to pentachlorocyclohexene and ultimately to the epoxide (Fitzloff et al. 1982).

2.6 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate due to maternal exposure during gestation and lactation. Relevant animal and in vitro models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in section 5.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both pre-natal and post-natal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Widdowson and Dickerson

1964; Foman et al. 1982; Owen and Brozek 1966; Altman and Dittmer 1974; Foman 1966). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and at various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults and sometimes unique enzymes may exist at particular developmental stages (Leeder and Kearns 1997; Komori 1990; Vieira et al. 1996; NRC 1993). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; West et al. 1948; NRC 1993). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

Limited information is available on the specific health effects resulting from HCH exposure in children. Generally, health effects observed in adults should also be of potential concern in children. Occasional deaths of children have been reported following ingestion of γ-HCH (Storen 1955). Although a causal relationship between exposure to γ-HCH and hematological effects in humans has not been established, there is one case report of hypochromic anemia and another of aplastic anemia in children exposed to γ-HCH by inhalation (Morgan et al. 1980; Rugman and Cosstick 1990). There are also sporadic reports of adverse effects of γ-HCH including convulsions in children after excessive topical application of γ-HCH (Lee and Groth 1977; Matsuope 1981; Ramchander et al. 1991; Telch and Jarvis 1982; Tenebien 1991). Neurological effects have been observed in immature animals exposed to γ-HCH. Weanling rabbits were more sensitive to lindane (γ-HCH) than young adults, as seen by increased mortality rates accompanied by excitement and convulsions after a single whole-body treatment with a 1% solution (60 mg lindane/kg) that was absorbed dermally (Hanig et al. 1976).

Alterations in cerebral levels of noradrenaline, serotonin, and dopamine were observed in rats treated intragastrically with a single dose of 20 mg/kg γ-HCH during the postnatal period (Rivera et al. 1991). Levels of noradrenalin were reduced in the mesencephalon. Concentrations of a serotonin metabolite were increased in the frontal cortex primarily on postnatal days 8 and 15 , but the results were not statistically significant. Levels of a dopamine metabolite were decreased in the mesencephalon, but statistical significance was only obtained on postnatal day 15 (+44%, $p<0.05$). According to the authors, earlier experiments demonstrated that higher doses of γ-HCH were required to increase serotonin in adult rats. Alterations in levels of brain dopamine, serotonin, gamma-aminobutyric acid (GABA), glutamate, glutamate decarboxylase, and noradrenaline were seen in various areas of the brains of female rat pups treated orally with 10 mg technical-grade HCH/kg/day for 60 days (Nagaraja and Desiraju 1994). Epileptiform seizures have been reported in male rats fed maternal milk for 12 days, 3 days after birth, from dams exposed to 20 mg γ-HCH/kg by gavage (Albertson et al. 1985).

No direct information is available regarding the effects of HCH on the developmental process in humans. However, developmental studies in animals indicated few effects from exposure to γ-HCH (Khera et al. 1979; Hassoun and Stohs 1996a; Srinivasan et al. 1991a); significant teratogenic effects were not observed (Khera et al. 1978). The proportion of embryos lost after implantation was increased after minks were treated with 1 mg/kg/day γ-HCH in their diet (Beard et al. 1997). An increase in the incidence of fetuses with extra ribs was reported in rats exposed to 20 mg/kg/day γ-HCH during gestation days 6-15 and in rabbits exposed during days 6–18 (Palmer et al. 1978a). However, the incidence of extra ribs within or just greater than the ranges recorded for the control groups, and therefore, may not be significant evidence of teratogenicity caused by exposure to γ-HCH (Hassoun and Stohs 1996a). β-HCH given to rat dams at 20 mg/kg/day during gestation caused increased fetal deaths within 5 days of birth (Srinivasan et al. 1991a). In another study, cadmium interacted with γ-HCH to cause significant embryotoxic and teratogenic effects in the developing rat fetus when administered together at a dosage that for either toxin alone is insufficient to cause any deleterious effects in development (Saxena et al. 1986).

β-HCH is lipophilic and accumulates in maternal adipose tissue and may be mobilized during pregnancy and lactation. HCH residues have been measured in human skin lipids (Dua et al. 1998) and in breastmilk (Dua et al. 1997; Czaja et al. 1997; Nair et al. 1996); HCH also crosses the placenta (Saxena et al. 1981). Its levels in placenta, maternal blood, and umbilical-cord blood were higher in cases of stillbirths than in live-born cases; however, many other organochlorine pesticides were present that could have contributed to stillbirths (Saxena et al. 1983). In a study in rats, γ-HCH has been reported to be transferred in the maternal milk and

to elicit neurological effects in neonates. Following intraperitoneal dosing of dams with γ-HCH on days 12–17 of gestation, $GABA_A$ receptors in rat fetuses were studied with radiolabeled t-butylbicyclophosphorothionate (TBPS), a ligand that binds to the $GABA_A$ receptor (Brannen et al 1998). Treatment with γ-HCH significantly reduced the TBPS binding affinity in fetal brainstems and it was concluded that the effect could potentially lead to abnormal brain activity, increased susceptibility to seizures, and behavioral effects. Also noted in the study, was reduced TBPS binding in brains of fetuses when compared to adults. In another study, lactating female rats were treated orally with a single dose of 6 mg/kg of γ-HCH on day 9 or 14, or 1 mg/kg on days 9–14 of lactation; the testosterone level of the male offspring was reduced 50% at puberty (day 60) when compared to the control group (Dalsenter et al. 1997a). When the offspring reached adulthood (day 140 postnatal), the relative testicular weight was significantly lower (Dalsenter et al. 1997). The number of sperm and spermatids was also significantly reduced.

Differences in oxidative effects have been observed in the testes of young versus mature rats, 15 and 90 days old respectively, following intrapertitoneal injection with 10 or 20 mg/kg technical-grade HCH (Samanta and Chainey 1997). Lipid peroxidation occurred to a greater extent in mature rats. However, the percent decrease in cytosolic superoxide dismutase activity was greater in young rats, which have increased baseline activity of the enzyme. Based on the findings of this study, it does not appear that young rats are at increased risk of oxidative testicular damage.

Although it is unknown whether the ability to metabolize HCH specifically differs between children and adults, some enzymes, which belong to the enzyme superfamilies involved in phase II HCH metabolism, are developmentally regulated in humans. The development of UDP-glucuronosyltransferase (responsible for glucuronide conjugation) depends on the enzyme isoform, but, in general, adult activity is attained by 6–18 months of age (Leeder and Kearns 1997). Development of sulfotransferase (responsible for sulfate conjugates) activity is also substrate specific and is usually earlier than UDP-glucuronosyltransferase. In fact, levels of some sulfotransferases may be greater during infancy and early childhood than during adulthood (Leeder and Kearns 1997). A series of enzymes are involved in the production of mercapturic acid conjugates: γ-glutamyltranspeptidase, glutathione S-transferase, cysteinyl glycinase, and N-acetyl transferase (Sipes and Gandolfi 1991). There are two superfamilies of N-acetyltransferase, and the N-acetyltransferase 2 superfamily has members that are developmentally regulated in humans. There is some N-acetyltransferase 2 activity in fetuses by 16 weeks of gestation. Infants up to 2 months of age have the slow metabolizer phenotype (there is a genetic polymorphism in this enzyme in adults). The adult

distribution of slow and fast metabolizer phenotypes is reached by 4–6 months of age and full adult activity is achieved at 1–3 years of age (Leeder and Kearns 1997).

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to hydrogen sulfide are discussed in Section 2.7.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by hydrogen sulfide are discussed in Section 2.7.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.9, Populations That Are Unusually Susceptible.

2.7.1 Biomarkers Used to Identify or Quantify Exposure to Hexachlorocyclohexane

There are few quantitative data to correlate levels of any of the HCH isomers in human tissue or fluids with environmental levels. A study in which children infected with scabies and their noninfected siblings were treated dermally with 1% lindane lotion found no correlation between the dose applied and the subsequent level of lindane in blood (Ginsburg et al. 1977). The blood level was also seen to diminish rapidly after application, with a half-life of 17.9 hours in infected children and 21.4 hours in noninfected children.

In contrast, β-HCH persists in the blood for a longer period of time than the other isomers. A study of workers in a lindane-producing factory found that levels of β-HCH in blood serum were higher than those of other isomers, and there was a significant correlation between serum levels of β-HCH and length of employment (Baumann et al. 1980). Studies of populations with general HCH exposure have consistently found the level of the β-isomer to be higher than those of the other isomers (Kashyap 1986; Nigam et al. 1986; Ramachandran et al. 1984). This is probably due to the greater tendency of β-HCH to persist and accumulate in the body while the other isomers are more rapidly metabolized or excreted. A survey of epidemiological studies involving workers occupationally exposed to "crude benzene hexachloride" as much as 10–15 years prior to sampling reported serum levels of 20–348 µg/L β-HCH (Morgan and Lin 1978). Unfortunately, none of the above studies specified exposure levels, so it is still questionable whether blood HCH levels can be used as biomarkers to quantify exposure.

There is also a direct correlation between HCH levels in the blood and human adipose tissue and semen (Baumann et al. 1980; Radomski et al. 1971a, 1971b; Szymczynski and Waliszewski 1981); concentrations of β-HCH in subcutaneous adipose tissues were found to be 300 times higher than blood levels (Baumann et al. 1980). Levels of β-HCH detected in skin lipids correlated with those found in human adipose tissue (Sasaki et al. 1991). Although exposure levels for the Japanese people in this study are not known, measuring β-HCH in skin lipids can be a rather easy method of determining relative levels or times of exposure among individuals. β-HCH and γ-HCH have also been found in samples of human maternal

adipose tissue, maternal blood, cord blood, and breast milk in women who were exposed to unknown levels of various organochlorine pesticides in Kenya (Kanja et al. 1992). The metabolites of γ-HCH have been detected in human urine (Angerer et al. 1981). However, such findings are not specific to γ-HCH exposure, and these findings could follow from exposure to both γ-HCH and a number of structurally related compounds.

2.7.2 Biomarkers Used to Characterize Effects Caused by Hexachlorocyclohexane

The individual isomers of HCH can be detected in the blood serum, urine, adipose tissue, and semen of exposed individuals. However, the concentrations measured in these biological tissues have not been exclusively correlated with the degree of adverse health effects observed.

Adverse effects such as neurophysiological and neuropsychological disorders and gastrointestinal disturbances have been reported in workers exposed to HCH during pesticide or fertilizer formulation. Nigam et al. (1986) and Kashyap (1986) reported that nonhandlers indirectly exposed and handlers directly exposed to HCH during pesticide manufacture and formulation were found to have mean serum levels of 0.27 ppm (nonhandlers) and 0.6 ppm (handlers) total HCH. As much as 60–100% of the total HCH measured in serum was β-HCH. The ranges of serum HCH levels measured in all exposed workers were 0.07–0.72 ppm β-HCH, 0.004–0.18 ppm α-HCH, 0–0.17 ppm γ-HCH, and 0–0.16 ppm δ-HCH. Both handlers and nonhandlers complained of paresthesia of the face and extremities, headache, and giddiness; other symptoms included malaise, vomiting, tremors, apprehension, confusion, loss of sleep, impaired memory, and loss of libido. Similar but less-severe effects were noted in 19 maintenance workers who visited the plant frequently. Serum HCH levels measured in these workers were 0.004–0.1 ppm α-HCH, 0.02-0.2 ppm β-HCH, 0–0.32 ppm γ-HCH, and 0–0.04 ppm δ-HCH. Kashyap (1986) also reported higher serum enzyme levels of alkaline phosphatase, lactate dehydrogenase, ornithine carbamyl transferase, γ-glutamyl transpeptidase, and leucine aminopeptidase and increased IgM in the handlers as compared with the nonhandlers and a control population of 14 workers with no occupational contact with HCH. Czegledi-Janko and Avar (1970) reported that γ-HCH blood levels of 0.024–0.16 ppm were associated with clinical symptoms including muscle jerking and variations in EEG in 37 workers exposed to γ-HCH in a fertilizer plant.

HCH and other organochlorine pesticides have been found in the blood serum of some individuals in a population of men attending an infertility clinic in Israel. Serum levels of organochlorine pesticides,

including γ-HCH, have been found in men with low sperm counts to be two times higher than that of fertile men (Pines et al. 1987). Maternal mean serum γ-HCH levels were reported to be higher in cases of premature delivery and spontaneous abortions than in controls (Saxena et al. 1980; Wassermann et al. 1982). Saxena et al. (1980) reported HCH levels of 69.3–550.4 ppb and γ-HCH levels of 30.8–113.6 ppb in the blood of women in India who had experienced spontaneous abortions or premature labor compared with blood HCH levels of 22.2–85.5 ppb and γ-HCH levels of 7.1–32.5 ppb in women who had undergone full-term pregnancy. Serum levels of a number of other pesticides including aldrin, DDE, DDT, and DDD were also found to be higher in cases of premature labor and spontaneous abortions. It was, therefore, not possible to establish a quantitative, causal relationship between the serum HCH levels and these adverse effects.

Blood serum levels of 1–17 ppb β-HCH were not found to be associated with the incidence of colorectal adenocarcinoma in 10 families (Caldwell et al. 1981). Serum levels of 0–49.5 ppb γ-HCH were not found to be associated with the occurrence of hematological syndromes such as pancytopenia, thrombocytopenia, plasma cell myoma, acute leukemia, chronic lymphocytic leukemia, and anemia in 103 patients (Traczyk et al. 1977).

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.8 INTERACTIONS WITH OTHER CHEMICALS

Guinea pigs maintained on diets deficient in vitamin C and protein showed altered γ-HCH metabolism and excretion. Vitamin C deficiency decreased the amount of γ-HCH and its metabolites excreted in the urine and increased the amount stored in the kidney (Chadwick et al. 1972c). Vitamin A supplements decreased HCH-induced toxicity in the rat testes, while deficiencies in vitamin A potentiated the toxicity (Pius et al. 1990).

Cadmium, which is known to inhibit hepatic drug-metabolizing enzymes in mammals, also inhibited the metabolism of γ-HCH in adult male Wistar rats exposed to the compound after short- and long-term pretreatment with cadmium (Chadwick et al. 1978b). Liver microsomal enzymes affected by exposure were γ-HCH dehydrogenase, γ-HCH dechlorinase, and hepatic cytochrome P-450 content. This action altered the profile of metabolites excreted in the urine. Cadmium may inhibit γ-HCH metabolism indirectly by

increasing levels of zinc and reducing levels of copper in the liver (Chadwick et al. 1978b). The addition of cadmium to the diet also increased the concentration of γ-HCH measured in the plasma and liver (Khanna et al. 1988). Cadmium also interacts with γ-HCH to cause significant embryotoxic and teratogenic effects in the developing rat fetus when administered together at a dosage that, for either toxin alone, is insufficient to cause any deleterious effects on development (Saxena et al. 1986).

A low-protein diet potentiated the effects of γ-HCH on reducing the weights of various organs in male rats (Khanna et al. 1990). Serum and liver lipid contents and cholesterol levels were increased in animals fed low-protein diets. The low-protein diet increased the levels of γ-HCH found in the various organ tissues.

The combined application of HCH (mixed isomers) and malathion to the skin of guinea pigs has been shown to produce a more severe toxicity when compared to animals treated with either insecticide alone. The results demonstrate an additive effect between concurrent dermal exposure to malathion and HCH, rather than a synergistic one (Dikshith et al. 1978).

γ-HCH exerts a neurotoxic effect following acute or chronic exposure. γ-HCH is a central nervous system (CNS) stimulant, whereas other isomers of HCH are CNS depressants (McNamara and Krop 1948a). Thus, neurotoxic effects excreted by γ-HCH are inhibited by α-, β-, and δ-HCH (McNamara and Krop 1948a, 1948b; Stein et al. 1980; Van Asperen 1954). "Raw γ-HCH," an intermediate in the production of γ-HCH, contains 16% α-HCH, 7% β-HCH, and 45% γ-HCH (Baumann et al. 1980). This may explain why occupational studies of workers exposed to HCH or γ-HCH manufacturing or application have reported contradictory results with respect to the neurotoxic effects observed.

The metabolism of γ-HCH can be altered by exposure to other chlorinated hydrocarbon insecticides such as DDT. Exposure to various chlorinated hydrocarbon insecticides, including γ-HCH, is thought to produce generalized nonspecific induction of microsomal enzymes. Induction of mixed-function oxidase activity by other chlorinated hydrocarbon insecticides stimulates the selective effect on the oxidative degradation of γ-HCH to the tetrachlorophenols and enhances its elimination in the urine (Chadwick and Freal 1972b). In addition, since HCH is hepatotoxic, therapeutic drops, which produce liver toxicity, such as acetaminophen may also enhance the symptoms of HCH exposure.

Single daily doses of 20 mg/kg γ-HCH in mice significantly reduced the convulsive threshold, as measured by the dose of pentylenetetrazol required to induce seizures 1–4 hours after treatment, but increased the

convulsive threshold 48 hours following treatment (Hulth et al. 1978). A dose of 50 mg/kg γ-HCH significantly increased the convulsive threshold 2, 4, and 10 days following dosing. A single dose of α-HCH significantly increased the convulsive threshold 3 and 24 hours after dosing and resulted in a significant 17% increase in brain levels of γ-aminobutyric acid (GABA) 24 hours after dosing.

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to HCH than most persons exposed to the same level of HCH in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or decreased excretion of HCH, or compromised function of target organs affected by HCH. Populations who are at greater risk, due to their unusually high exposure to HCH, are discussed in Section 5.7, Populations With Potentially High Exposure.

People with excoriated (peeling) skin exhibited higher levels in blood of γ-HCH following dermal exposure than those with normal skin (Ginsburg et al. 1977). It was not known if there were any increased toxic effects to individuals with excoriated skin. It is also not known if children are unusually susceptible to the toxic effects of HCH, but anecdotal evidence suggests that it should not be used on infants and young children. The potential hazards of using γ-HCH preparations on infants and young children are underscored by the fact that the very young have a large surface area-to-volume ratio, possibly less efficient hepatic detoxification abilities, and are more likely to lick treated skin (Kramer et al. 1980). Therefore, the use of γ-HCH as a scabicide on infants and very young children, especially those who have very little body fat, has been discouraged (Telch and Jarvis 1982).

Evidence suggests that pregnant women should excercise extreme caution in their exposure to γ-HCH (Ginsburg et al. 1977; Kramer et al. 1980; Solomon et al. 1977a). Refer to Section 2.6 for more detailed explanation. In pregnant animals and humans, γ-HCH crosses the placenta. HCH and γ-HCH body tissue levels have also been associated with premature labor and spontaneous abortions (Rasmussen 1980; Saxena et al. 1980, 1981a, 1981b; Wassermann et al. 1982). However, no causal relationship has been established between blood and tissue levels of γ-HCH and premature termination of pregnancy.

Nair (1996) demonstrated that there is a significant bioconcentration of the α-, β-, and γ-isomers of HCH in the breastmilk of mothers exposed to technical-grade HCH.

People with lowered convulsion thresholds due to epilepsy (treated or untreated), cerebrovascular accidents, or head injuries may be at greater risk of the central nervous system effects of γ-HCH toxicity and may suffer increased risk of or severity of seizures (Kramer et al. 1980; Matsuoka 1981). Those individuals suffering from malnutrition (e.g., low protein, low fiber, and low vitamin intake) may be more susceptible than the general public to the toxic effects of γ-HCH (Rasmussen 1987). Individuals with liver and/or kidney disease may be at risk because of compromised detoxification mechanisms in the liver and impaired excretory mechanisms in the kidney. Additionally, individuals with existing or suspected immunodeficiencies may be at risk because HCH isomers may enhance immunosuppression.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to hexachlorocyclohexane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to hexachlorocyclohexane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following text provides specific information about treatment following exposures to hexachlorocyclohexane: Ellenhorn and Barceloux 1988.

2.10.1 Reducing Peak Absorption Following Exposure

When a large amount of HCH has been swallowed, emetics have been used to induce vomiting. One of the problems with inducing vomiting is that the insecticidal form of HCH is often dissolved in an organic solvent, which presents an aspiration hazard. Activated charcoal can also be used to decrease gastrointestinal absorption. To avoid skin absorption after exposure, clothing should be removed, and the skin should be washed with water and mild soap (Ellenhorn and Barceloux 1988). There are no known methods for reducing absorption following inhalation exposure.

2.10.2 Reducing Body Burden

The traditional methods of increasing elimination or decreasing distribution (e.g., dialysis, diuresis, and hemoperfusion) are not useful because of the high volume of distribution of HCH into adipose tissue (Ellenhorn and Barceloux 1988). HCH accumulates in adipose tissue following all routes of exposure.

However, peritoneal dialysis may be required if rhabdomyolysis (muscle necrosis) leads to myoglobinuria and kidney shutdown (Sunder Ram Rao et al. 1988).

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

Possible mechanisms of action of HCH on some of the target organs have been described. In the nervous system, γ-HCH is thought to interfere with the GABA system by interacting with the GABA-A receptor-ionophore complex at the picrotoxin binding site (Portig and Schnorr 1988; Rivera et al 1991; Sunol et al. 1988). Thus, the seizures caused by γ-HCH can be antagonized by GABA-A mimetics; diazepam is the anticonvulsant of choice (Ellenhorn and Barceloux 1998). Phenobarbital and/or phenytoin or fosphenytoin may be used if seizures are uncontrollable (HSDB 1998). Use of anticonvulsants (especially in children and other susceptible individuals) should include careful monitoring of hypotension, respiratory depression, and the need for endotracheal interbation. In the liver, γ-HCH is thought to produce oxidative stress by inducing oxidative enzymes such as cytochrome P-450 and depleting hepatic glutathione content (Barros et al. 1988, 1991; Srinivasan and Radhakrishnamurty 1988; Videla et al. 1991). Another possible mechanism for hepatic toxicity is increased lipid metabolism (Ravinder et al. 1990; Srinivasan and Radhakrishnamurty 1988). It is possible that interfering with these mechanisms can decrease the toxicity of HCH.

2.11 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of hexachlorocyclohexane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of hexachlorocyclohexane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

Most of the literature reviewed concerning the health effects of inhaled α-, β-, γ-, or δ-HCH in humans consists of case reports of individuals occupationally exposed or exposed in the home by a γ-HCH vaporizer. The predominant route of exposure in occupational studies is presumed to be inhalation, although dermal exposure is also likely. The health effects in humans associated with ingested HCH are reported primarily in case studies in which individuals ingested pesticide pellets or therapeutic lotions containing γ-HCH to control scabies. Information concerning the health effects of HCH in humans following dermal exposure is limited to case studies of individuals who have misused therapeutic lotions containing γ-HCH to control scabies and head and body lice. The duration and level of exposure to HCH generally cannot be quantified from the information presented in these reports. In addition, the case study reports in humans are limited because concomitant exposure to other toxic substances or other substances present in the atmosphere may have occurred.

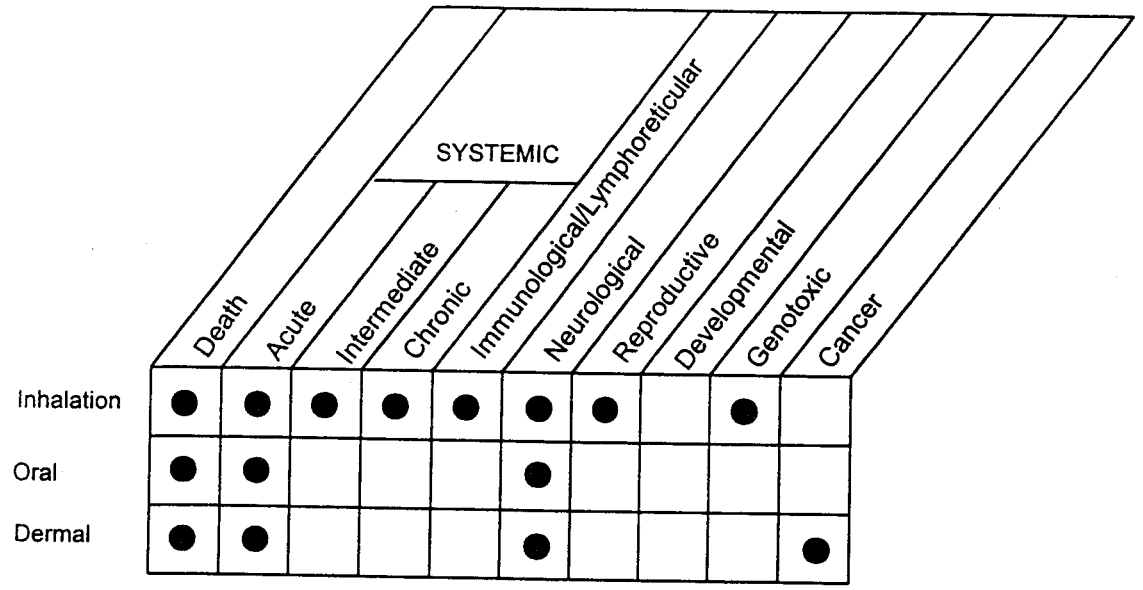
Limited information was found regarding the health effects of lindane following inhalation exposure in animals. The health effects of α-, β-, γ-, and δ-HCH following oral exposure have been well documented in a variety of species. Limited information is available concerning the health effects of technical-grade HCH and lindane following dermal exposure.

γ-HCH is the isomer most thoroughly tested in intermediate- and chronic-duration studies. The carcinogenic effects of technical-grade HCH and α-, β-, and γ-HCH have been examined, but the carcinogenic potential of δ-HCH has not been as well studied. Studies on the long-term effects of dermal exposure to γ-HCH are inadequate for the determination of carcinogenicity status.

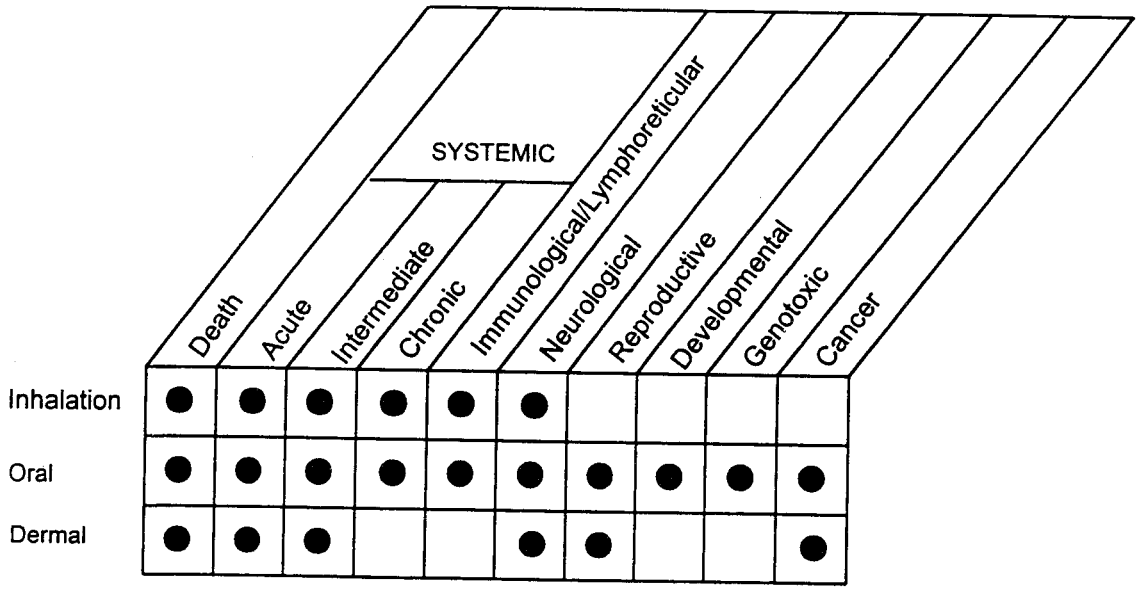
2.11.1 Existing Information on Health Effects of Hexachlorocyclohexane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to HCH are summarized in Figure 2-7. The purpose of this figure is to illustrate the existing information concerning the health effects of HCH. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct

FIGURE 2-7. Existing Information on Health Effects of HCH



Human



Animal

● Existing Studies

comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

2.11.2 Identification of Data Needs

Acute-Duration Exposure. Occasional case reports are available for humans who have had adverse health effects, including irritation of the nose and throat and death, from excessive inhalation exposure from γ-HCH vaporizers (Conley 1952; Loge 1965). Oral exposure to large amounts has resulted in a few human deaths (Storen 1955; Sunder Ram Rao et al. 1988) and adverse neurological, musculoskeletal, and renal effects (Munk and Nantel 1977; Sunder Ram Rao et al. 1988). When applied dermally, γ-HCH has also been shown to have adverse effects such as pulmonary and epicardial petechiae, aplastic anemia, and rashes in a few humans (Davis et al. 1993; Fagan et al. 1981; Rauch et al. 1990). The level of exposure in the human studies generally cannot be quantitated because the information is derived from anecdotal case reports. Therefore, there is little reliable information in humans associating dose with effect. Such information might allow investigators to establish thresholds for systemic toxicity due to acute exposure.

Information on health effects (death and neurological) following acute inhalation of γ-HCH in animals (Ullmann 1986b; Klonne and Kintigh 1988; Oldiges et al. 1980) is limited. Neurological effects following acute inhalation exposure to γ-HCH have included excitation, sedation, ataxia, and spasms (Ullmann 1986b). Acute inhalation studies for the other HCH isomers and technical-grade HCH are not available. No acute inhalation MRL was developed because of insufficient data. Additional acute inhalation data are needed for all isomers, e.g., threshold, dose-response, and target organ. This information is necessary for determining levels of significant human exposure to hexachlorocyclohexane and the associated effects following exposure. An acute oral MRL of 0.01 mg/kg has been developed from data on increased kindling acquisition following exposure to γ-HCH (Joy et al. 1982). An acute oral MRL of 0.2 mg/kg/day for β-HCH has been developed based on ataxia in mice (Cornacoff et al. 1988). Other acute oral studies in animals exposed to γ-HCH have reported death in rats (Gaines 1960) and mice (Liu and Morgan 1986), increased hepatic microsomal mixed-function oxidase activity in mice (Oesch et al. 1982), and degeneration of renal tubular epithelia in rats (Srinivasan et al. 1984). Oral exposure to β-HCH has resulted in an increase in heptatic cytochrome P-450 levels and renal tubular degeneration in rats (Ikegami et al. 1991b; Srinivasan et al. 1984), and exposure to technical-grade HCH has resulted in hepatic focal necrosis, fatty changes, and enzyme activation and renal hemorrhage (Phillip et al. 1989; Ravinder et al. 1989; Dickshith et al. 1990). Additional studies which examine systemic effects (e.g., cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, and

renal) following acute oral exposure to all HCH isomers would be helpful. Acute dermal studies in rats are available on γ-HCH and technical-grade HCH (Dikshith et al. 1991c; Gaines 1960). Acute dermal exposure of rats to γ-HCH (Gaines 1960) or of guinea pigs to technical-grade HCH (Dikshith et al. 1978) was associated with lethality. Additional acute dermal data in animals are needed, for example, threshold, dose-response, and target organs. This information is necessary for determining levels of significant human exposure to HCH and the associated health effects following dermal exposure.

Intermediate-Duration Exposure. Information on human health effects of repeated exposure to HCH is available from studies of occupationally exposed individuals (Kashyap 1986); no information is available on the effects of repeated oral or dermal exposure in humans. EEG abnormalities and increased liver enzymes have been observed in factory workers involved in the production of technical-grade HCH (Kashyap 1986). The exact duration and level of exposure in the human studies are often not provided in the studies. Such information would allow investigators to determine health effects associated with known levels of exposure.

Intermediate-duration inhalation studies of γ-HCH have been performed in rats with mortality reported (Klonne and Kintigh 1988). Inhalation of 603 mg/m^3 γ-HCH for 4 hours or 5 mg/m^3 for 90 days has not resulted in adverse respiratory, hematological, hepatic, or renal effects in rats (Oldiges et al. 1983). However, the data are insufficient for developing an intermediate-inhalation MRL. Additional intermediate-inhalation data in animals are needed, e.g., threshold, dose-response, and target organs. This information is necessary for determining levels of significant human exposure to hch and the associated health effects following inhalation.

Intermediate-duration oral studies have been performed in animals. Oral γ-HCH did not affect the hematological parameter in rats (Suter 1983) and dogs (Rivett et al. 1978). Decrease in blood cell numbers was observed in rats fed β-HCH (Van Velsen et al. 1986) and technical-grade HCH (Joseph et al. 1992c). Hepatic effects in animals following γ-HCH exposure included hypertrophy, necrosis, and cancer (Hanada et al. 1973; Ito et al. 1973; Suter 1983). Hepatic effects in animals, following exposure to β-HCH, included cellular hypertrophy and necrosis (Ito et al. 1973; Van Velsen et al. 1986; Hanada et al. 1973); α-HCH induced hepatic effects included enzyme activation, hypertrophy, necrosis, and cancer (Barros et al. 1991; Hanada et al. 1973; Ito et al 1973). Hepatic effects from technical-grade HCH exposure in animals included changes in enzyme activities and enlargement of hepatocytes, nuclear pyknosis, and vacuolation (Dikshith et al. 1989a, 1991a; Fitzhugh et al. 1950; Karnik et al. 1981; Joseph et al. 1992b). Renal effects from γ-HCH exposure included nephritis, accumulation of protein droplets, hypertrophy, and necrosis (Suter 1983);

nephritis was observed following α-HCH exposure (Fitzhugh et al. 1950). Exposure to β-HCH has resulted in calcinosis and nephritis (Van Velsen et al. 1986; Fitzhugh et al. 1950); technical-grade HCH exposure has resulted in nephritis and tubular necrosis (Dikshith et al. 1991a; Fitzhugh et al. 1950). Two MRLs have been derived for intermediate-duration oral exposure in animals. An intermediate oral MRL of 0.0006 mg/kg/day for β-HCH has been developed based on hepatic effects in rats (Van Velsen et al. 1986). An intermediate oral MRL for γ-HCH of 0.00001 mg/kg/day has also been developed based on immunological effects in mice (Meera 1992).

Intermediate-duration dermal studies have been performed in rabbits, guinea pigs, and rats; some deaths were observed following exposure to γ-HCH (Brown 1988). There are limited data pertaining to systemic effects (e.g., increased respiratory rate and wheezing, hepatic hypertrophy, and basophilic renal tubules) and neurological effects (e.g., hyperactivity, ataxia, and convulsions) in rats following intermediate-duration dermal exposure to γ-HCH (Brown 1988). Death and systemic effects (e.g., hepatic hypertrophy and fatty degeneration and renal tubular necrosis) have been observed in rats (Dikshith et al. 1991c); hepatic hypertrophy and enzyme activation were observed in guinea pigs (Dikshith et al. 1978) following intermediate-duration dermal exposure to technical-grade HCH. Additional intermediate-dermal data in animals are needed, e.g., threshold, dose-response, and target organs. This information is necessary for determining levels of significant human exposure to HCH and the associated health effects following dermal exposure.

Chronic-Duration Exposure and Cancer. Controlled epidemiological studies have been conducted in humans exposed to HCH, but are few in number and limited in scope. Hematological effects have been observed in persons exposed to γ-HCH in the workplace via the inhalation and/or dermal route (Brassow et al. 1981; Jedlicka et al. 1958). A number of case reports are available from individuals who had exposure to γ-HCH in the home, during the handling of the pesticide, or from a nearby formulating plant (Danopoulos et al. 1953; Friberg and Martensson 1953; Gewin 1939; Loge 1965; Mendeloff and Smith 1955). Effects that have been described in these case reports include hematological effects including granulocytopenia, aplastic anemia, paramyeloblastic leukemia, and pancytopenia. Chronic-duration oral studies are not available for humans.

No chronic-duration inhalation studies in animals are available for any isomer. Altered renal excretions and hepatic hypertrophy and have been observed in chronic oral studies on rats with γ-HCH (Amyes et al. 1990). A chronic oral MRL of 0.008 mg/kg/day for α-HCH has been developed based on hepatic effects in rats

(Fitzhugh et al. 1950). Chronic dermal studies in animals are not available. Since there are insufficient data to develop inhalation, and dermal, chronic-duration MRLs, further data from the inhalation and dermal routes are needed (e.g., threshold, dose-response, and target organs). This information is necessary for determining levels of significant human exposure to HCH and the associated health effects. However, the need for dermal studies is not a priority as data on skin absorption can be used to calculate equivalent oral doses.

Use of γ-HCH pesticides by farmers was associated with a 50% increased risk of non-Hodgkin's lymphoma (Blair et al. 1998). However a causal relationship could not be determined due to confounding effects such as use of other pesticides. Limited chronic dermal data in humans are available (Davis et al. 1993), but chronic oral data in humans are not available. There are no inhalation studies in animals. Several chronic toxicity/carcinogenicity bioassays have been conducted in animals following oral exposure to technical-grade HCH and α-, β-, γ-, and δ-HCH (Hanada et al. 1973; Ito et al. 1975; Karnik et al. 1981; Kashyap et al. 1979; Munir et al. 1983; NCI 1977; Thorpe and Walker 1973; Wolff et al. 1987). Chronic dermal exposure to technical-grade HCH caused liver cancer in mice (Kashyap et al. 1979). However, the results were not useful in determining carcinogenic potential because of limitations of these studies, such as testing only one dose and the potential for oral ingestion. 2,4,6-Trichlorophenol, a metabolite of γ-HCH, may be responsible for some or all of the carcinogenic activity observed in mice. This metabolite has been classified by EPA as a group B2 carcinogen. Pentachlorocyclohexene epoxide, a metabolite of γ-HCH that has been identified in the liver of rats, may also be responsible for the carcinogenic effects of γ-HCH. Cancer classifications of several HCH isomers have been made by the U.S. Department of Health and Human Services (DHHS) and the EPA. EPA has classified technical-grade HCH, α-HCH, β-HCH, and δ-HCH as B2, B2, C, and D, carcinogens, respectively (EPA 1998a). γ-HCH has not been assigned a cancer classification by EPA. Additional carcinogenicity information would not be needed at this time. DHHS has classified γ-HCH and other HCH isomers as "reasonably anticipated to be human carcinogen" in the 8th Report on Carcinogens (DHHS 1998). The International Agency for Research on Cancer (IARC) has classified HCH isomers as Group 2B, possibly carcinogenic to humans.

Genotoxicity. HCH did not produce chromosomal aberrations in humans exposed primarily by inhalation (Kiraly et al. 1979). Dominant lethal mutations occurred in mice orally exposed to technical-grade HCH (Lakkad et al. 1982). Increased frequency of polyploid cells occurred in rats exposed orally to α-HCH (Hitachi et al. 1975). Information on the genotoxic effects of γ-HCH is also obtained from *in vitro* studies. Gene mutations were observed in bacteria treated with γ-HCH (with and without metabolic activation) (Moriya et al. 1983; Nagy et al. 1975). γ-HCH was not mutagenic in yeast (Shahin and von Borstel 1977) or

algae (Kar and Singh 1979a). Results of chromosomal aberration tests in γ-HCH-treated hamster cells was questionable (Ishidate and Odashima 1977). Technical-grade HCH produced chromosomal aberrations in cultured human lymphocytes (Rupa et al. 1989d) but did not produce cytogenetic effects in Chinese hamster cells (Murli 1990) or unscheduled DNA synthesis in rat hepatocytes (Cifone 1990). In general, the available information suggests that α-, β-, and γ-HCH may have some genotoxic potential; however, the evidence is not conclusive. Further testing in clastogenicity and genotoxicity tests *in vivo* would be valuable.

Reproductive Toxicity. The only available human data are from one study on hormone levels in pesticide workers in which increases in the levels of serum luteinizing hormone were noted following exposure to γ-HCH for 8 years (Tomczak et al. 1981) . There are no inhalation data in animals for any HCH isomer. Anti-estrogenic properties were found in female rats given γ-HCH by the oral route (Chadwick et al. 1988). Female rabbits treated orally with γ-HCH had a reduced ovulation rate (Lindenau et al. 1994). No adverse effects were reported in a three-generation study in rats treated with 5, 10, or 20 mg/kg/day γ-HCH (Palmer et al. 1978b). Decreased weight gain was observed in the mid- and high-dose group. Oral exposure of rats and mice to β- or technical-grade-HCH has resulted in degeneration of male reproductive organs and sperm abnormalities (Dikshith et al. 1991a; Gautam et al. 1989; Nigam et al. 1979; Pius et al. 1990; Roy Chowdhury and Gautam 1990; Van Velsen et al. 1986), and ovarian atrophy was observed in rats exposed to β-HCH for 13 weeks (Van Velsen et al. 1986). Similar effects were also observed in reproductive organs of rats following dermal treatment with technical-grade HCH for 120 days (Prasad et al. 1995). The reproductive effects on guinea pigs after dermal exposure to technical-grade HCH (100–500 mg/kg/day) have also been investigated (Dikshith et al. 1978). Testicular hypertrophy and atrophy and complete inhibition of spermatogenesis were observed in the guinea pigs. Studies via the inhalation and dermal routes would provide information regarding the reproductive effects of HCH in animals for these exposure routes and could be useful in the assessment of potential reproductive effects in humans.

Developmental Toxicity. Information regarding the developmental effects of HCH in humans was not found for any exposure routes. There are no inhalation data in animals for any isomer. No adverse developmental effects of γ-HCH from oral exposure have been found in rats or rabbits (Khera et al. 1979; Palmer et al. 1978a; Seiler et al. 1994) or from exposure to technical-grade HCH in mice (Dikshith et al. 1990). Alterations in neurotransmitter levels were noted in suckling rats treated once with γ-HCH by gavage (Rivera et al. 1991). No effects on embryonic development were seen in rabbits treated orally with γ-HCH (Seiler et al. 1994). However, decrease in fetal weight, fetal thymic weight, and placental weight have been reported in mice exposed to a single oral dose of γ-HCH on day 12 of gestation (Hassoun and Stohs 1996a). Alterations

in neurotransmitter levels were observed in female rat pups treated orally with technical-grade HCH (Nagaraja and Desiraju 1994). No data on the developmental effects of α-, β-, or δ-HCH were located for the oral or dermal route and there is no information for dermal exposure to technical-grade HCH. Additional developmental studies in animals exposed to α-, β-, or δ-HCH by all three routes would provide information concerning the possible fetotoxic and teratogenic effects in animals, which might be relevant to humans.

Immunotoxicity. A statistically significant increase (approximately 18%) in IgM has been reported in individuals occupationally exposed to technical-grade HCH (Kayshap 1986). The HCH isomer concentrations in serum showed a 10-fold increase when compared to the control. There are no oral or dermal data in humans. Also, there are no inhalation or dermal data in animals. Depressed antibody response to *Salmonella* antigens was reported in rats (Dewan et al. 1980) and rabbits (Desi et al. 1978) exposed to γ-HCH via the oral route. γ-HCH exposure has been shown to result in thymus cortex atrophy, suppressed bone marrow cellularity, erythrocyte precursors, and granulocyte-macrophage progenitor cells in mice (Hong and Boorman 1993). Based on immunological effects of γ-HCH on components of cell- and humoral-mediated immunity in mice, an intermediate oral MRL has been developed (Meera et al. 1992). Decreased lymphoproliferative responses to T-cell mitogens were observed in mice treated by the oral route with β-HCH (Cornacoff et al. 1988). No immunological effects were observed in rats treated with β-HCH by the oral route for 13 weeks (Van Valsen et al. 1986). There is no immunotoxicity data for technical-grade HCH. The biological significance of increased immunoglobulin levels remains to be established. In addition, exposure to technical-grade or γ-HCH may also affect the immune system in humans (Kashyap 1986) and animals (Desi et al. 1978; Dewan et al. 1980). Further studies on all isomers using all three routes of exposure would be useful in the assessment of potential immunotoxic effects in humans.

Neurotoxicity. Exposure to γ-HCH has been shown to be associated with neurological effects in both humans and animals (Czegledi-Janko and Avar 1970; Kashyap 1986; Van Velsen et al. 1986). Paresthesia has been reported in workers exposed via the inhalation or dermal routes (Fonseca et al. 1993; Kashyap 1986). Abnormal EEG patterns have also been noted in workers (Czegledi-Janko and Avar 1970). Seizures and coma have been observed in individuals who have ingested large amounts of γ-HCH (Davies et al. 1983; Harris et al. 1969; Munk and Nantel 1977; Nantel et al. 1977; Powell 1980; Starr and Clifford 1972; Storen 1955). Convulsions have been reported in children following dermal application of γ-HCH (Tenebein 1991; Ramchander et al. 1991). Neurological effects including sedation, restlessness, excitation, and ataxia were seen in rats exposed by inhalation to γ-HCH for 4 hours (Ullmann 1986b). Mice exposed via the inhalation route to γ-HCH in a chronic-study did not display any neurotoxic signs (Klonne and Kintigh 1988).

Convulsions have been observed in rats and mice following oral exposure to γ-HCH (Arisi et al. 1994; Barron et al. 1995; Gilbert 1995; Gilbert and Attia et al. 1991; Joy et al. 1982; Mack 1995; Martinez et al. 1991; Vendrell et al. 1992a; Martinez and Martinez-Conde 1995; Wooley and Griffith 1989). Less serious effects such as decreased myelin and enzyme activity in brain and reduced tail nerve conduction velocity were observed in rats following oral exposure to γ-HCH (Muller et al. 1981; Serrano et al. 1990a). Oral exposure of mice and rats to β-HCH has resulted in lateral recumbency, coma, and reduced tail nerve conduction velocity (Cornacoff et al. 1988; Muller et al. 1981; Van Velsen et al. 1986). Rats and mice exposed orally to technical-grade HCH experienced convulsions, increased motor activity, and variations in neurotransmitter levels (Anand et al. 1991; Dikshith et al. 1991a; Gopal et al. 1992; Kashyap et al. 1979). Neurological effects were not observed in rats following oral exposure to α-HCH (Muller et al. 1981). Information is available on the neurotoxic effects of α-, β-, and γ-HCH in experimental animals following acute-duration oral exposure (Tilson et al. 1987; Tusell et al. 1987; Woolley and Griffith 1989) and intermediate-duration oral exposure (Desi 1974; Muller 1981; Van Velsen 1986). An acute oral MRL of 0.01 mg/kg/day for γ-HCH was derived based on neurological effects (increased kindling acquisition) in rats (Joy et al. 1982). Also, an acute oral MRL of 0.2 mg/kg/day for β-HCH was developed based on ataxia in mice (Cornacoff et al. 1988). Studies in animals have substantiated the neurological symptoms resulting from dermal application of γ-HCH. Effects in rats included sedation, spasms (Ullmann 1986a), tremors, and convulsions (Brown 1988). Neurochemical and neurophysiological studies in animals exposed via the oral route would provide useful information regarding the mechanisms of HCH-related neurotoxic effects. Because an MRL could not be developed for inhalation and dermal exposures, additional studies for all isomers for these two exposure routes would be useful.

Epidemiological and Human Dosimetry Studies. Information on the adverse health effects of HCH in groups of humans comes from reports of occupationally exposed individuals (Brassow et al. 1981; Jedlicka et al. 1958; Kayshap 1986). Adverse health effects include EEG abnormalities, increased liver enzymes, and changes in hematological parameters. Limitations inherent in these studies include unquantified exposure concentrations and durations and concomitant exposure to HCH mixtures and other chemicals and pesticides. The few industrial surveys and studies of exposed individuals generally reported blood levels of HCH following exposure and the health effects associated with these levels (Czegledi-Janko and Avar 1970). However, the reported blood levels of HCH have not been quantitatively correlated with ambient HCH levels or health effects. Studies that provide information correlating exposure levels with body levels of HCH would allow investigators to monitor humans for exposure, including populations living near hazardous waste sites. Well-conducted studies would be helpful in determining and quantifying the effects of inhalation, oral,

or dermal HCH exposure on human health including neurological, hematologic, and hepatic effects. However, considering the magnitude of the needed studies and lowered likelihood of exposure in present day society, the value of such studies is questionable.

Biomarkers of Exposure and Effect

Exposure. Methods exist for the analysis of HCH in blood and urine (Angerer et al. 1981). Thus, biological monitoring for exposure to HCH is possible by measuring the levels of HCH in the blood or urine. In an occupational study, abnormal EEG changes were found to correlate with blood levels of γ-HCH (Czegledi-Janko and Avar 1970). Measurements of γ-HCH represent short-term exposure because it is metabolized and excreted rapidly. Due to its high lipid solubility and persistence, β-HCH level represents long-term exposures. β-HCH has been measured in numerous human tissues and is the isomer that is consistently detected at the highest concentration (Baumann et al. 1980; Kashyap 1986; Morgan and Lin 1978; Nigam et al. 1986; Ramachandran et al. 1984). However, the reported blood levels of HCH have not been quantitatively correlated with ambient HCH levels. Methods that measure the levels of HCH metabolites in urine are not specific enough to detect exposure to HCH alone. More information could be provided by studies designed to correlate biomarkers of exposure with exposure levels.

Effect. No biomarkers of effect, specific for HCH, have been identified in the literature. Nonspecific biomarkers of effect include EEG abnormalities, increases in liver enzymes, hematological effects, seizures and convulsions, neuropsychological, and gastrointestinal effects (Nigam et al. 1986; Kasyup 1986). Muscle spasms and EEG abnormalities have also been observed in workers exposed to γ-HCH (Czegledi-Janko and Avar 1970). High levels of HCH and other organochlorine insecticides have been detected in men with low sperm counts and in women who miscarriage or deliver prematurely (Pines et al. 1987; Saxena et al. 1980; Saxena et al. 1980; Wassermann et al. 1982). No quantitative correlation can be made between body levels of HCH and adverse health effects based on the existing data. Studies quantitatively correlating HCH exposure with body levels of HCH and the occurrence of specific adverse health effects would be useful for monitoring populations possibly exposed near hazardous waste sites. Studies designed to identify specific biomarkers of effect for HCH would be useful.

Absorption, Distribution, Metabolism, and Excretion. Information is available to assess the extent and rate of HCH absorption following oral exposure in animals and humans (Ahdaya et al. 1981; Albro and Thomas 1974; Turner and Shanks 1980). High blood concentrations of γ-HCH have been demonstrated in a number of acute poisoning cases in which humans were exposed to γ-HCH as the result of ingestion (Berry et al. 1987). Animal studies indicate that γ-HCH is readily absorbed from the gastrointestinal tract (Ahdaya et al. 1981). Both *in vivo* and *in vitro* studies that evaluate dermal absorption of γ-HCH in humans are available (Dick et al. 1997a, 1997b). However, absorption of HCH via inhalation can only be inferred from toxicity studies and studies assessing the distribution and excretion of γ-HCH. No quantitative information is available to assess the rate and extent of inhalation absorption in humans or animals. Additional data concerning the absorption of HCH in animals may provide information to assist in characterizing absorption of HCH in humans.

Information on the distribution of HCH isomers in humans is inferred from case studies, clinical studies, and industrial surveys (Baumann et al. 1980; Nigam et al. 1986; Siddiqui et al. 1981a). Air concentrations of α-HCH, β-HCH, and γ-HCH have been found to be associated with blood serum levels in workers (Baumann et al. 1980). HCH isomers have been detected in the adipose tissue of workers (Baumann et al. 1980). γ-HCH was detected in the cerebral spinal fluid of a young boy following ingestion of γ-HCH (Davies et al. 1983). γ-HCH was detected in brain tissue collected during the autopsy of an infant who was treated with a whole-body application of γ-HCH lotion (Davies et al. 1983). The distribution of HCH in animals following oral exposure has been well documented (Chand and Ramachandran 1980; Eichler et al. 1983; Srinivasan and Radhakrishnamurty 1983b). γ-HCH and β-HCH were found to be primarily stored in the fat of rats after acute oral exposure. Except in the brain, β-HCH accumulates in tissues to a greater degree than γ-HCH. α-HCH has been shown to accumulate preferentially in the white matter of the brain (Portig et al. 1989). Data exist on the rate and overall distribution of HCH in animals following dermal application. In guinea pigs, the accumulation of γ-HCH in the brain was greater than in the blood following acute dermal exposure (Solomon et al. 1977a, 1977b).

The metabolism of γ-HCH has been studied in mice and rats (Chadwick and Freal 1972a; Chadwick et al. 1978a; Engst et al. 1979; Kujawa et al. 1977). Researchers have identified the primary metabolites (di-, tri-, and tetrachlorophenols) in humans, rats, and mice. In humans, this information is obtained from urinary excretion studies in which individuals were occupationally exposed to γ-HCH (Angerer et al. 1983; Engst et al. 1979). *In vitro* studies using rat liver microsomes have helped to delineate the major metabolic processes and have demonstrated the formation of a reactive epoxide that may be indicative of similar processes in

other mammals and humans (Fitzloff and Pan 1984). Investigations have not been conducted to examine the epoxide formation *in vivo* or its role in inducing mutagenic and carcinogenic effects. Extensive metabolic studies have been conducted in animals, and adequate studies exist identifying major metabolites in the tissues and urine (Macholz et al. 1982a, 1982b; Chadwick and Freal 1972a; Kujawa et al. 1977). Multiple detoxification pathways have been delineated (Chadwick et al. 1978a, 1981; Kujawa et al. 1977). Further information on the possible role of epoxide formation in carcinogenesis *in vivo*, as well as its rate of formation under various conditions, would be useful.

Information from occupational studies and studies in which γ-HCH was used as a therapeutic lotion is available to conclude that humans excrete HCH, principally as metabolites, in urine, breast milk, and semen (Angerer et al. 1981). Urinary excretion of γ-HCH metabolites by humans has been documented (Angerer et al. 1983). The primary urinary metabolites of γ-HCH are chlorophenols. Quantitative information also exists to conclude that the primary route of HCH excretion in animals, following oral exposure, is urine (Chadwick et al. 1985). There are no inhalation studies that have examined the excretion of HCH. In male rats treated dermally with radiolabeled γ-HCH, radiolabel was detected in the urine (Bosch 1987a).

Comparative Toxicokinetics. Evidence is available to suggest that rats and humans absorb HCH and store the isomers primarily in the fat and other body tissues (Chand and Ramachandran 1980; Eichler et al. 1983; Srinivasan and Radhakrishnamurty 1983b). Similar metabolites have been identified in the urine of exposed individuals and treated rodents, and in both, the primary route of excretion is the urine (Angerer et al. 1981; Chadwick et al. 1985).

Exposure to γ-HCH has been shown to be associated with neurological effects in both humans and animals (Czegledi-Janko and Avar 1970; Kashyap 1986; Van Velsen et al. 1986). The available human and animal data also suggest that HCH isomers may affect the blood system. In addition, HCH isomers may also affect the immune system in humans (Kayshap 1986) and animals (Desi et al. 1978; Dewan et al. 1980). Further studies are not needed at this time.

Methods for Reducing Toxic Effects. Seizures caused by γ-HCH can be antagonized by GABA-A mimetics; diazepam is the anticonvulsant of choice (Ellenhorn and Barceloux 1988). The available data

indicate some ways in which peak absorption of HCH might be reduced following oral or dermal exposure (Ellenhorn and Barceloux 1988). Intestinal absorption can be reduced with activated charcoal, while washing with soap and water can decrease skin absorption. There are no known methods for reducing absorption following inhalation exposure.

Because of the high volume of distribution of HCH into adipose tissue, traditional methods of increasing elimination or decreasing distribution are not useful. Development of methods to enhance the excretion of HCH from adipose tissue, while minimizing toxicity would be beneficial in reducing the body burden.

There is some information on the mechanism (see Section 2.4) for the toxic effects of HCH on the brain (e.g., interference with the GABA system) (Abalis et al. 1985; Casida and Lawrence 1985; Lawrence and Casida 1984) and liver (e.g., disruption of oxidative defense mechanisms) (Barros et al. 1988, 1991; Srinivasan and Radhakrishnamurty 1988; Videla et al. 1991). Further studies in these areas might be helpful for developing methods for reducing toxic effects.

Children's Susceptibility. Limited data are available on the health effects of HCH on exposed children.

It has been demonstrated that weanling rabbits were more sensitive to lindane than young adults, as seen by increased mortality rate and associated excitement and convulsions after treatment (Hanig et al. 1976). However, there is no actual evidence that children are more sensitive to the neurotoxicity of γ-HCH. It would be useful to follow up on the weanling rabbits study and conduct additional studies on immature postnatal animals as an experimental model. Data needs relating to developmental effects are discussed above in developmental toxicity section. Replicating the Dalsenter et al. (1997) study on lactational exposure and adult testosterone levels should be a priority. There is inadequate experimental evidence to determine if pharmacokinetics of HCH in children are different from adults. There is no experimental evidence to indicate whether metabolism of HCH or its mechanism of action is different in children compared with adults. Generally, it would be difficult to have data on the metabolism and mechanism of action of HCH in children (except in accidentally exposed children) to determine whether children are more vulnerable than adults to adverse health effects from exposure to HCH. There are no biomarkers of exposure or effect that have been validated in children or adults exposed as children. There is no data to determine whether there are any interactions with other chemicals that are unique to children or whether interactions observed in adults also occur in children. Although HCH is shown to have some genotoxic potential, it is not known whether parental exposure to HCH may affect children via parental germ cells, or whether HCH may indirectly affect

the fetus during maternal exposure. Additional data is needed to determine the potential for genotoxicity in germ cells and adverse developmental effects.

Child health data needs relating to exposure are discussed in 5.8.1 Data Needs: Exposures of Children.

2.11.3 Ongoing Studies

The following studies involving γ-HCH are ongoing:[a]

INVESTIGATOR	INSTITUTION	SUBJECT	SPONSORED BY
Loch-Caruso, R.	University of Michigan	Lindane modification of uterine muscle	National Institute of Environmental Health
Wooley, D.	University of California, Davis, CA	Physiological effects of acute and chronic exposure to environmental toxicants	U.S.D.A

[a]Information based on data identified in CRIS/USDA (1998) database.